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ENDOCARDIAL FIBRO-ELASTOSIS: A STUDY OF EIGHT CASES*

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Endocardial fibro-elastosis is an unusual disease most often seen at autopsy in infants dying within the first year of life. The condition is generally considered to be a developmental disturbance of unknown origin and should be classified with the congenital malformations of the heart. The anatomical lesion consists of a yellowish white thickening of the endocardium which manifests a very definite predilection for the left side of the heart. Frequently this endocardial abnormally is only mural, but may sometimes be associated with marked valvular deformities. The term "fetal endocarditis" has been used in referring to these two lesions. This usage is unfortunate both because the term connotes an inflammatory process and also because endocarditis usually implies valvular vegetative disease. The concomitant occurrence of the mural and valvular defects has caused considerable confusion in the past, many authors failing to distinguish any differences between the two lesions. Gross[1] painstakingly reviewed the entire subject in 1941 and aided in the establishment of anatomical diagnostic criteria. He raised several objections to the traditional interpretation of the lesion as inflammatory and added one case to the literature.

A survey of isolated case reports demonstrated the need of a correlative study, clinical and pathologic, with special emphasis upon an accurate description of the endocardial and valvular microscopic lesions. Such a study should include a statistically significant number of cases. With this in mind it was believed that a review of a series of 8 cases

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TABLE I

Summary of Cases of Endocardial Fibro-elastosis

Case number	Autopsy number	Age at death	Sex	Clinical picture	Duration of symptoms	Mural lesion	Valvular deformity	Heart weight
								gm.
1	CM 6162	5 mos.	F	Not available	Not available	Left ventricle	None	107
2	CM 5952	3½ mos.	M	Sudden onset of generalized cyanosis, shivering, and restlessness	2 days	Left ventricle	None	60
3	A–44–214	4 mos.	F	Several episodes of fainting, sudden collapse with respiratory difficulty and death	10 days	Left auricle Left ventricle	None	72
4	A–45–136	9 mos.	F	Episodes of nausea, vomiting and diarrhea, sudden dyspnea, and cyanosis	14 days	Left auricle Left ventricle	None	105
5	A–49–84	7 days	M	Dyspnea and cyanosis since birth	7 days	Left ventricle	Mitral and aortic	44
6	MPA46–5A	23 days	M	Sudden cyanosis and crying; mitral systolic and diastolic murmurs since birth	23 days	Left auricle Left ventricle	Mitral and aortic	33
7	MPA46–3	Stillborn	F	Hydramnios (8 l.) at 5 mos.' gestation; twin birth and sac of this child abnormal	Stillborn	Left ventricle	None	12
8	MPA46–5	5 mos.	M	Sudden onset of irritability and gurgling cough	3 days	Left auricle Left ventricle	Mitral	53

which have been autopsied at Syracuse University Medical Center would be of value.

Some of the data relevant to these cases are outlined in Table I.

Sex and Age Incidence

In the series herein described there were 4 male and 4 female infants. Study of other reports indicates that the disease does not manifest any definite sex preference. The 4 cases described by Farber and Hubbard[2] were all in males, while there was one male and one female in the 2 instances added by Weinberg and Himelfarb.[3] A review of isolated case reports discloses a fairly equal sex distribution.

Table I demonstrates the rather marked variability of age incidence. The youngest child in this study was case 7, one of fraternal twin girls, who were stillborn after 5 months of gestation. A hydramnios was present (estimated 8 l.) and the sac of the affected twin was stated to be the larger. Case 4, the oldest in our series, was 9 months of age at the time of death and had manifested symptoms for only 2 weeks. This infant has a longer duration of life than any of the cases we have reviewed from the literature. We cannot, however, be certain that occasional children with this cardiac malformation do not go on to adulthood. Some evidence for this belief is found in a paper by Comeau[4] who has described endocardial sclerosis in 2 middle-aged individuals who were without evidence of arteriosclerosis.

Excluding case 7, the average age at death for the infants reported here was 4 months.

Clinical Picture

It will be noted that the majority of the infants in our series were stated to have been normal at birth, and the sudden onset of symptoms and the extremely short duration of life thereafter would appear to be very striking characteristics of this disease. Cyanosis, noted in 4 of the 8 infants, was the most common presenting sign. Generalized in some, and limited solely to the lips in others, this cyanosis was accentuated by effort, such as crying. Irritability, anorexia, and dyspnea frequently were present.

Physical examination of these children revealed the usual manifestations of congestive heart failure. Mitral systolic and diastolic murmurs were heard in case 6 and valvular lesions were present at autopsy. No record of the clinicians' findings were available in case 5. In case 8 a murmur had not been heard, but the child died at the age of 5 months after a 3-day illness. This infant had been examined in routine periodic visits by a competent pediatrician.

It is of interest that no example of an ante-mortem diagnosis of this condition can be found in the literature. In a previously apparently healthy baby, the sudden onset of cyanosis, fainting, and dyspnea, with or without cardiac murmurs, should suggest that endocardial fibro-elastosis be considered in the differential diagnosis.

The duration of life following onset of symptoms was extremely short in our series. Excluding cases 1 and 7, the average survival time following symptoms was 10 days. Failure of both ventricles occurred and marked pulmonary edema was present uniformly as a cause of death.

Gross Pathologic Findings

Cardiac enlargement was present universally, the heart weights varying from two and one-half to four times that expected for the age. Left ventricular hypertrophy, which was described as moderate or marked in every case, accounted for this increased weight. Had electrocardiographic studies been made, left-sided preponderance should have been evident. The heart was globular in every instance. As can be seen in Figure 1, the endocardium appeared thickened, gray, and somewhat opaque. Only the left side of the heart was involved in our series, the distribution being auricular and ventricular in 4 instances, while the ventricle alone was affected in the remaining 4. It will be noted that the involved chambers always were thickened diffusely. Bellet and Gouley[5] and Philpott[6] have described endocardial defects similar to this in association with other cardiac anomalies. Excluding the valvular malformations, there were no other cardiac defects in our cases.

Valvular deformities were present in 3 instances and the appearance of the cusps and leaflets was similar in all. The aortic valves in cases 5 and 6 showed cusps which were swollen, nodular or slightly wrinkled, and glistening white (Figs. 2 and 3). No vegetations were seen. With involvement of the mitral valves in cases 5, 6, and 8, the leaflets showed similar changes but with extension downward to incorporate the chordae tendineae so that the papillary muscles appeared almost continuous with the fused, thickened leaflets (Fig. 5). This thickening and fusion of the leaflets extended to the free margin where a distinct rolled border was apparent. There was marked stenosis of the valvular orifice with hypertrophy and dilatation of the auricle. Surface vegetations were not present in our cases. Gross involvement of other viscera was limited to pulmonary edema, hepatic congestion, and mesenteric lymphadenopathy. The last was presumably associated with the ascites which was invariably present.

Microscopic Pathologic Findings

The tissue was fixed in 10 per cent formalin and absolute alcohol and stained with hematoxylin and eosin, Weigert's elastic tissue stain, and Best's carmine stain for glycogen.

The mural endocardial subdivisions as outlined by Maximow and Bloom[7] will be followed in the ensuing description. These authors stated that the endocardium is composed of three distinct layers: a thin endothelial lining, a subendothelial coat containing varying proportions of collagen, elastic and smooth muscle fibers, and a subendocardial layer. The last is made up of loose connective tissue with varying numbers of blood vessels and nerves.

The microscopic alterations in all of our cases were generally similar. The endothelial cells were unusually prominent, occasionally appearing piled up as if attempting the formation of a second layer. The absolute increase in endocardial thickness was due to the great number of orderly arranged elastic fibers with a slight excess in the usual amount of collagen (Fig. 4). These elastic fibers generally were fragmented and delicate in the more superficial parts of the endocardium while they were coarser and took a dark stain in the zone adjacent to the subendocardium (Fig. 6). The subendocardial-myocardial junction usually was ill defined, and numerous degenerating myocardial fibers were noted in that area. Special stains demonstrated large amounts of glycogen within these degenerating myocardial cells (Fig. 7), while very little glycogen was seen in the remainder of the myocardium. The endocardium was avascular except for the slightly dilated vessels normally seen at its junction with the myocardium. It is to be emphasized that no evidence of active inflammation was seen in this zone in any case.

Myocardial changes were noted only in the papillary muscles. Fibrosis, calcification, and hydropic degeneration were prominent within these structures (Fig. 8). We believe that such degenerative phenomena could effect serious functional derangement of the papillary muscles, going on, perhaps, to some degree of valvular insufficiency. Hypertrophic muscle nuclei were present in all of the hearts.

Study of the valvular structure in the cases with the so-called endocarditis revealed a general similarity to normal valvular architecture (Figs. 9 and 10). The swelling in the affected valves was due to a proportionate increase in the number of round and spindle-shaped cells, and in basophilic interstitial tissue. Blood and lymphatic channels were prominent in these swollen leaflets (Fig. 10). Elastic tissue stains on involved valves disclosed dark-staining material within the ground plate

of their substances. These condensations consisted of basophilic reticular fibers and stood in sharp contrast to the positive-staining elastic tissue of the endocardium (Fig. 11). Rare elastic fibers were identified running longitudinally beneath the endothelium of both valve surfaces. No inflammatory cellular infiltrations were seen.

Sections of other organs disclosed the usual evidences of visceral hyperemia, particularly within the lungs and liver. No abnormalities within the elastic tissue of the viscera or vascular system were noted.

Discussion

The factors concerned in the formation, maintenance, and disintegration of elastic tissue are not well understood. Haas[8] thoroughly reviewed the existing literature in 1939 and concluded that it was a neglected subject and that only a beginning had been made in its study. Majority opinion holds the fibroblast to be the precursor of both elastic and collagen fibers. Elastic tissue, however, does not react to stimuli in the same manner as does the parent fibroblast or the sibling collagen fiber. Factors known to influence both growth and disintegration of elastic tissue are age, heredity, inflammation, endocrine glandular activity, mechanical stresses, and blood supply. It is not within the scope of this paper to elaborate upon these factors and the reader is referred to the study by Haas for a complete review. Brief mention will be made here of one or two factors since it is believed that these may be implicated in the pathogenesis of endocardial fibro-elastosis.

Much attention has been directed toward inflammation as the agent responsible for both the endocardial and valvular alterations. Congenital syphilis,[9] maternal influenza,[2] and bronchitis[10] have each been ascribed a rôle. In none of our cases was there any evidence of maternal illness during gestation, and Wassermann tests of the blood, when available, were always negative. Maternal cardiac disorders[2] and rheumatic heart disease have been implicated also. In connection with the latter, Pocock's case[11] has been cited as evidence. He made a clinical diagnosis of rheumatic fever in a premature infant whose mother was suffering from that disease at the time of delivery. Since the infant recovered, the case lacks confirmation. Ferguson[12] reported an instance of rheumatic fever in a newborn, but the diagnosis appears to have been made on insufficient clinical evidence. This child's mother had suffered a severe attack of rheumatic fever persisting from the second month of pregnancy until delivery. Kissane and Koons[13] described a newborn with painful swollen joints and abnormal heart sounds, born of a mother with active rheumatic fever. The child lived to age 9 and, at autopsy, a typical buttonhole mitral valve with shortened chordae tendineae and

thickened papillary muscles were noted. Aschoff bodies were found within the mitral leaflets and right auricular wall. This case constitutes the most convincing evidence for the existence of intra-uterine rheumatic fever, but bears no resemblance either grossly or microscopically to any of our cases.

In general, inflammation of both the acute exudative and chronic productive types is characterized by extreme degeneration and disappearance of elastic fibers. The only exception to this statement is found in the work of Bunting[14] who studied the new formation of elastic tissue in adhesions between serous membranes and in myocardial scars. His study may have some bearing on endocardial fibro-elastosis since Bunting demonstrated that abundant elastic tissue develops in adhesions between serous membranes and in scars which are subject to alternation of tension and relaxation. The endocardium, which is subjected to such stress during the cardiac cycle, certainly fulfills this criterion. This idea necessitates postulating that endocardial fibro-elastosis is in reality scar tissue following a preceding inflammatory process. It would appear unlikely that an infection would be so specialized in its selectivity as to be limited to the endocardial elastic tissue of the left side of the heart. Furthermore, we cannot regard microscopic fibrosis and calcification within the papillary muscles as evidence for a pure inflammatory genesis of the lesion, although others[2,15] have done so. It is our thought that these changes constitute reaction to tissue degeneration, the cause of which remains obscure at present. Haas[8] has pointed out that elastic tissue is known to exhibit quantitative and qualitative variations in local and general distribution. Moreover, these are influenced by hereditary and congenital factors and vary somewhat with certain constitutional types and states of endocrine activity. Thus, the hypothesis seems more reasonable to us that endocardial fibro-elastosis may come about due to an inborn derangement of mesenchymal tissue, to be placed in a category with the other congenital cardiac malformations.

Because of the nearly complete absence of elastic tissue from the valvular lesions, we suspect that the valvular process is a separate, although related, anomaly. Evidence for some association is the fact that, whereas endocardial fibro-elastosis may occur frequently without concomitant valvular involvement and distortion, the reverse does not appear to be true. Since the affected leaflets are devoid of any inflammatory reaction and because of the absence of vegetations we feel that the term fetal endocarditis is not only inappropriate, but actually misleading. Fibro-elastosis, implying as it does similar mural and valvular lesions, likewise must be considered as faulty. Endocardial dysplasia,

which may or may not include the valves, is suggested as a more accurate designation for the combined lesions.

Left ventricular failure appears to be the cause of death in children with endocardial fibro-elastosis. Weinberg and Himelfarb[3] have offered one explanation: they postulated interference with emptying of the arterioluminal vessels into the ventricle because of the thick fibroelastic layer. This acts by constriction of their orifices causing dilatation and stasis within the intramyocardial capillaries. Ventricular anoxia and failure apparently follow. Vascular dilatation was not a feature of our cases except at the endocardial-myocardial junction. We were more impressed with the degenerative changes within the papillary muscles and suggest interference with their function as an additional factor in the production of ventricular failure. There is still another mechanism which may be concerned in the production of heart failure in these cases. It is conceivable that the thick, rigid endocardium may act in a manner somewhat analogous to that of chronic constrictive pericarditis. In this instance the endocardial abnormality would tend to limit both the diastolic filling and the systolic expulsion of the involved ventricle.

Summary

Eight new cases of endocardial fibro-elastosis have been analyzed in respect to both clinical and pathologic features. The evidence indicates that this condition is a developmental disorder of mesenchymal tissue, to be classified with the congenital cardiac malformations. Both the mural and valvular lesions are of a non-inflammatory nature. The term endocardial dysplasia is suggested to replace fetal endocarditis and endocardial fibro-elastosis, both of which have misleading connotations.

We wish to express our appreciation to Dr. J. Howard Ferguson for his many valuable suggestions; to Dr. Arthur E. Harris of Auburn, New York, for permission to use case 6; and to Miss Stella Zimmer of the Department of Photography for the illustrative materials.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 136

FIG. 1. Case 1. Globular heart showing thickened, opaque, left ventricular endocardium. Myocardial hypertrophy is present.

FIG. 2. Case 5. Swollen, slightly nodular, aortic leaflets. The left ventricle has a thickened endocardium and hypertrophic myocardium.

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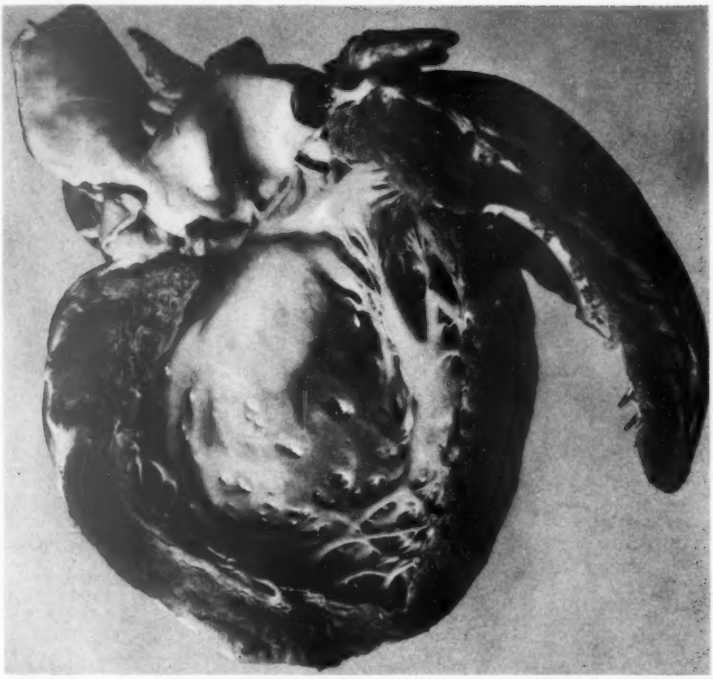

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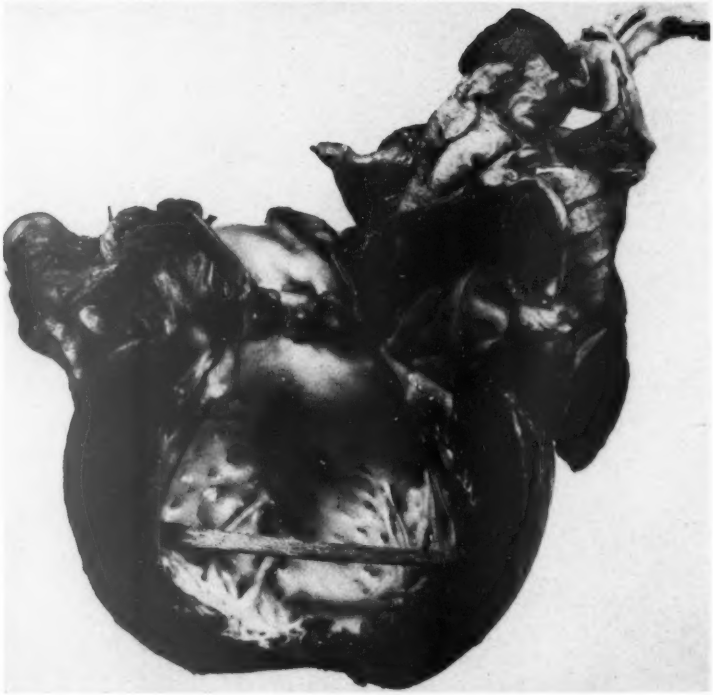

Prior and Wyatt Endocardial Fibro-elastosis

PLATE 137

FIG. 3. Case 6. Thickened, fused, glistening white, aortic leaflets. The endocardial defect is apparent.

FIG. 4. Case 3. Entire left auricular wall, emphasizing the absolute increase in the endocardial thickness. × 25.

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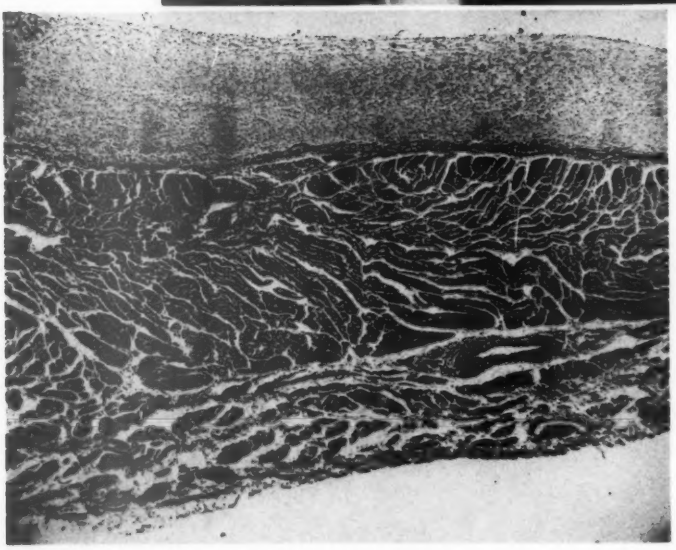

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Prior and Wyatt Endocardial Fibro-elastosis

PLATE 138

FIG. 5. Case 6. Mitral valve showing thickened cusps and involvement of the chordae tendineae. The papillary muscles appear to attach directly to the leaflet at the right margin.

FIG. 6. Case 3. Elastic tissue stain of the endocardium, demonstrating the superficial fine elastic fibrils, and the more coarse dark-staining elastic tissue adjacent to the myocardium. × 200.

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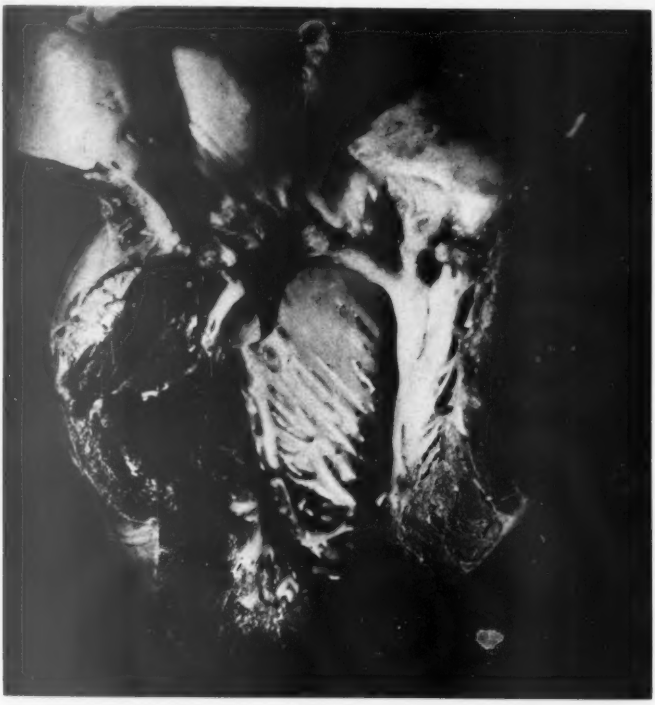

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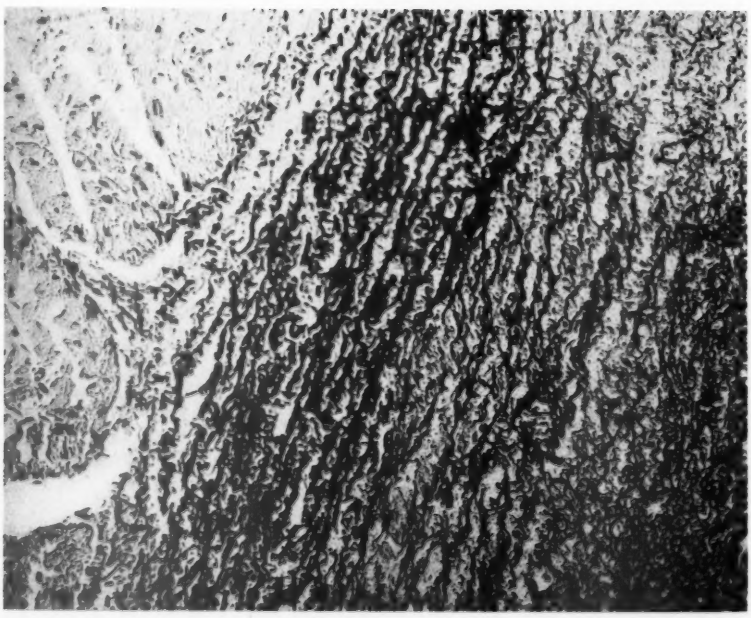
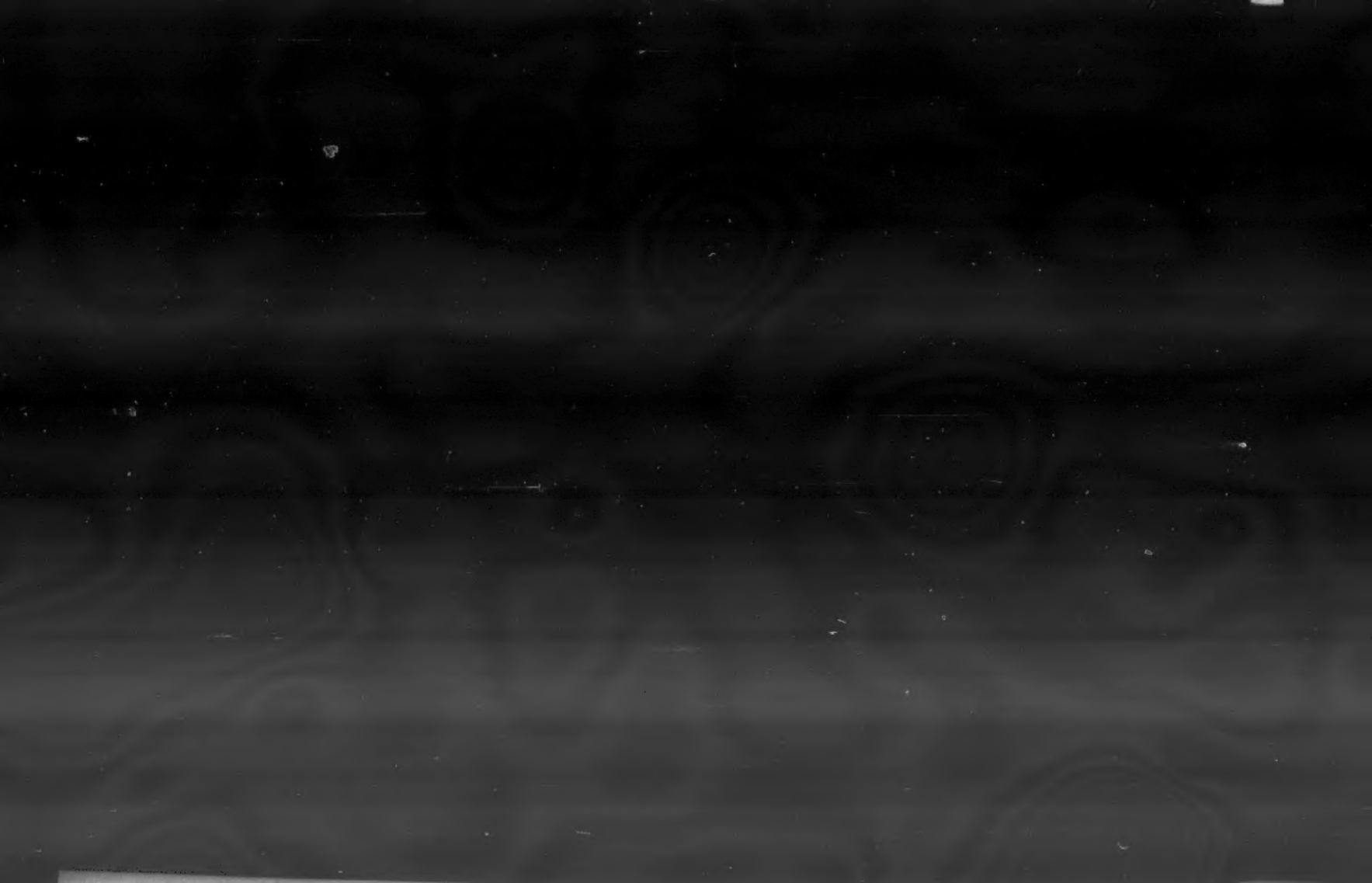


Prior and Wyatt Endocardial Fibro-elastosis

PLATE 139

FIG. 7. Case 3. Glycogen stain of the endocardium. The dark-staining material represents glycogen deposition within degenerating myocardial fibers adjacent to the endocardium. × 200.

FIG. 8. Case 5. Left ventricular papillary muscle with fibrosis and calcification. × 25.



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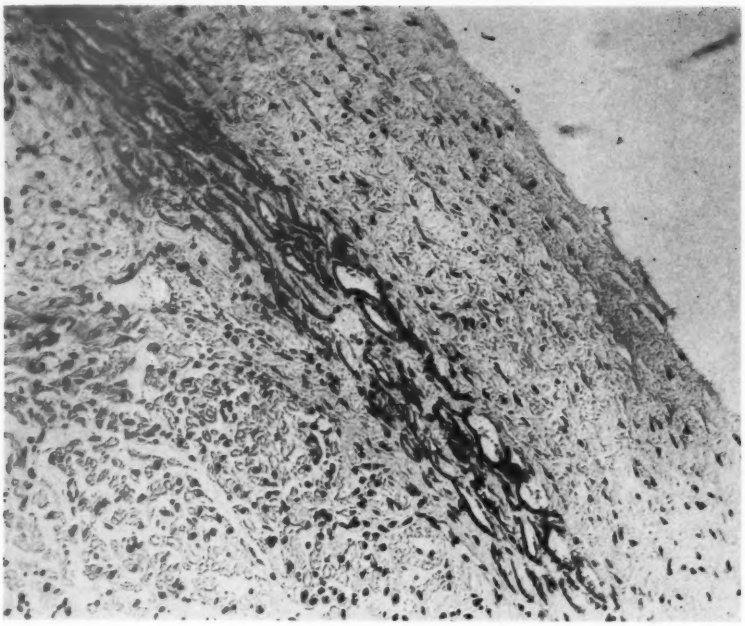

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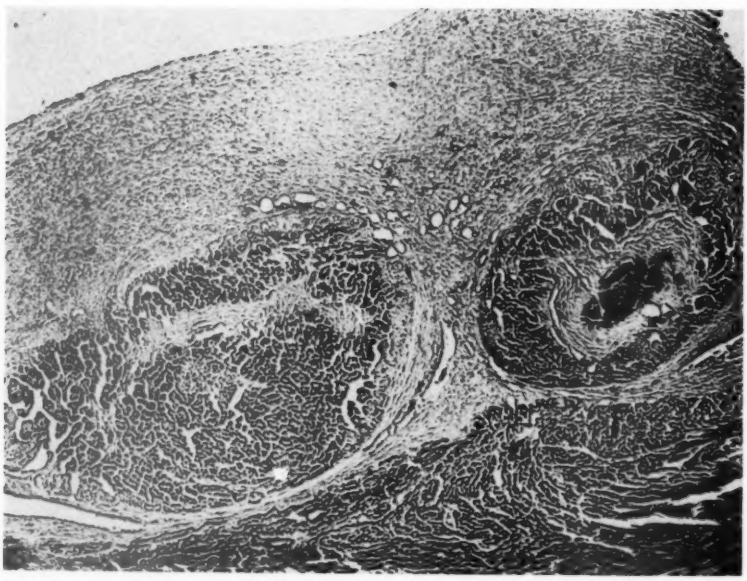


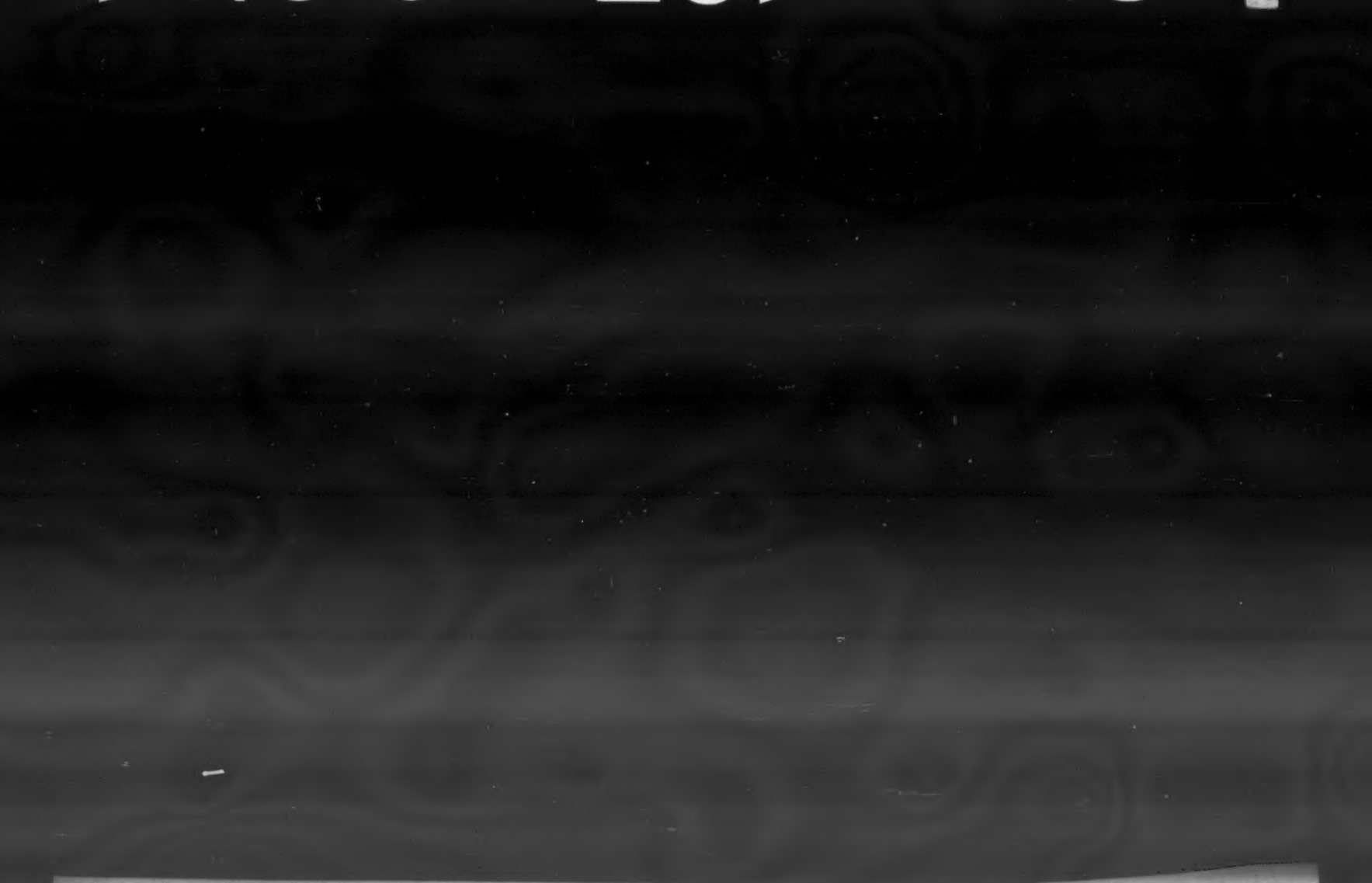
Prior and Wyatt Endocardial Fibro-elastosis

PLATE 140

FIG. 9. Case 5. Normal pulmonary valve leaflet for comparison with Figure 10. × 25.

FIG. 10. Case 5. Aortic valve leaflet with the so-called fetal endocarditis. Of note is the proportionate increase in the tissue normally present. Dilated vascular spaces are apparent. × 25.

FIG. 11. Case 5. Elastic tissue stain of an affected mitral leaflet. The darker staining material within the valve represents condensation of reticular fibers and contrasts sharply with the endocardial elastic fibers below. × 25.



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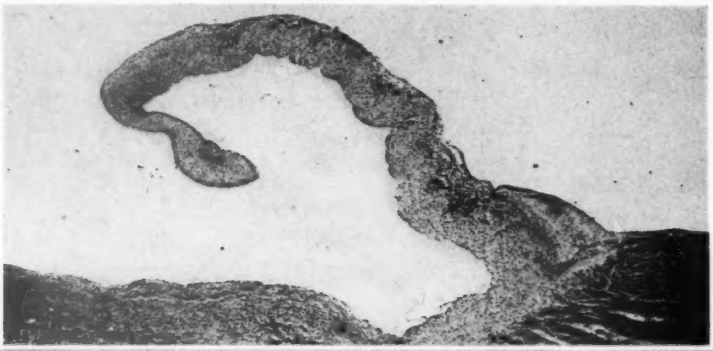

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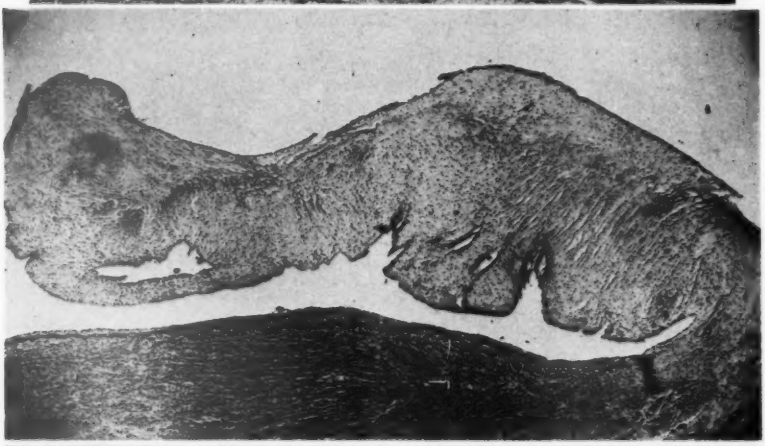

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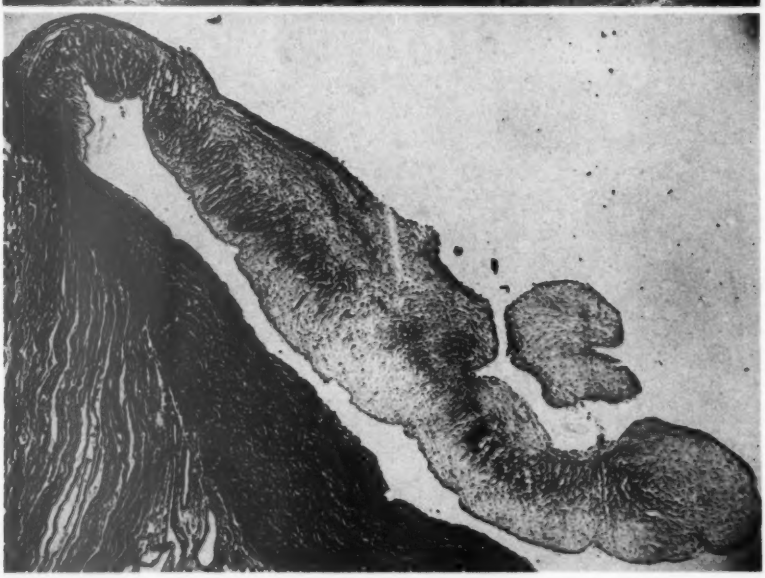

Prior and Wyatt Endocardial Fibro-elastosis

THE INTERRELATION OF ELASTIC TISSUE AND CALCIUM IN THE GENESIS OF ARTERIOSCLEROSIS *

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According to Wells,[1] "the disorganization of arterial walls with advancing age presents features which seem to indicate that this is, primarily, only the natural behavior of colloidal membranes." A study of the age changes in the "elastic colloid" might therefore be expected to yield important information on the genesis of arteriosclerosis. Yet such an approach has received relatively little attention among investigators. Instead, major effort has been put on the study of lipid metabolism and its relation to the formation of atheromatous plaques.

Extending Wells' line of reasoning, it was thought that there would be similarities between age changes in arterial walls and the processes of ageing of tissues generally; in 1944 we[2] had observed, as in other soft tissues, a progressive increase in the calcium content of the media of the aorta with increasing age. These changes precede the formation of intimal plaques and probably condition their genesis. At that time we reviewed the evidence in favor of the concept that medial changes are, in large part, responsible for the subsequent formation of intimal atheromata. Subsequently Yater, Traum, Brown, Fitzgerald, Geisler, and Wilcox[3] have presented further evidence, based on a study of human autopsy material, indicating that the deposition of lipids in the intima is secondary rather than primary. Experiments with cockerels[4] also have indicated that medial degeneration is the primary lesion in coronary arteriosclerosis.

Our recent study[5] of ageing processes in coronary arteries has demonstrated further that calcification is related to changes in elastic tissue. In these arteries there appears to be a splitting and fragmenting of the internal elastic lamella accompanied by calcification of the fragments. It would thus appear that two processes common to ageing tissues occur in arterial walls: changes in the state of certain colloidal elements as indicated by Wells,[1] and calcification of these elements.

The present report is a continuation of these studies; a comparison has been made of the changes in elastic tissue and calcium in some arteries in which atheromatous plaques are commonly found with those

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† Deceased.

in arteries in which atheromata occur relatively infrequently. By such a comparison it may be possible to characterize further the process of elastic tissue calcification and to determine its relation to the formation of intimal plaques.

Material and Method

We have studied the hepatic, renal, and iliac arteries in approximately 140 human autopsy cases. The vessels were fixed in 10 per cent formalin in absolute alcohol and sections prepared as in previous studies.[2,5] In each instance a micro-incinerated section, one stained for elastic tissue with resorcin-fuchsin, and a third stained with hematoxylin and eosin were compared. Grading of the severity of calcification, 1 to 4 plus, was carried out as in previous investigations.[2,5]

Table I

Distribution of Cases by Age Groups *

Age group	Number of cases		
	Hepatic	Renal	Iliac
0–19 yrs.	6	9	8
20–39 yrs.	13	9	12
40–49 yrs.	17	19	20
50–59 yrs.	19	21	17
60–69 yrs.	35	38	34
70–79 yrs.	25	31	29
Over 80 yrs.	13	13	11
Total	128	140	131

* We wish to express our appreciation for the assistance given by Dr. John A. Saxton, Jr., Director of the Snodgras Laboratory, St. Louis City Hospital, in making available many of the specimens studied in these investigations.

The distribution of cases is shown in Table I. A comparison of the changes in elastic tissue and calcium over the span of years shown in Table I was carried out with each of the arteries listed. In most instances it was possible to compare the three vessels in the same individual, although in occasional cases all three arteries were not available for study. A comparison was made also of the rate of calcification of the arteries presented in this and previous reports. In addition, an attempt was made to correlate the severity of these processes with some of the diseases commonly encountered in an autopsy series; namely, diabetes, hypertension, tuberculosis, and cancer.

Results

Alterations in Elastic Tissue and Calcium with Age

Hepatic Artery

During the first 2 decades of life the internal elastic lamella lay in juxtaposition to the endothelial lining of the hepatic artery. The ex-

ternal lamella consisted of one or several parallel wavy bands. The media was essentially devoid of elastic elements until the latter part of the second decade when scattered fine filaments of elastic tissue were found in the media. These appeared to arise from either lamella, but in most instances these filaments were more numerous near the internal elastic layer. Micro-incinerated preparations showed a thread-like line of calcium along the inner surface of the vessel. The external lamella showed no evidence of calcification and the media presented only a cellular distribution of calcium corresponding to the nuclear elements. The pattern was essentially the same in the succeeding 2 decades except that there was a progressively increasing number of elastic filaments in the media. The latter showed no particular tendency to calcify up to age 40 (Figs. 1 and 2).

No general thickening of the intima was noted prior to the fifth decade of life. In occasional specimens there were focal areas of duplication of the internal elastica toward the intima; the latter were thinner than the parent membrane, which could be easily identified. In such areas deeper, fine, wavy, elastic fibrils also extended into the media and broke off continuity with the internal elastica. The external elastica was composed of numerous parallel wavy fibrils from which fragments of elastic elements also extended into the media as well as into the adventitial fat. It was during the fifth decade that foci of calcification appeared in the media and duplicated the pattern of the elastic elements in this layer; most of this calcification was toward the intimal half of the media and was generally about 1 plus in intensity.

After the fifth decade the hyaline thickening of the intima and duplication of the internal elastic lamella became progressively more marked. The fragmentation and granulation of elastic elements which appeared to arise at the base of this membrane also became progressively more intense and gradually extended deeper into the media. An increasing number of fragments also extended from the external lamella. While there was diffuse hyaline thickening of the intima, plaques were encountered rarely. Calcification of the elastic elements in the media became progressively more severe with advancing age, but seldom exceeded 3 plus in intensity. The external elastica calcified only rarely, and in such exceptional cases it occurred in areas adjacent to fragments of elastic tissue. Intact elastic membranes calcified only slightly. Calcification was much more severe in elastic fibrils and fragments; the wormy and granular pattern in elastic tissue preparations was frequently duplicated in the micro-incinerated specimens (Figs. 3 and 4).

There appeared to be no change from the average degree of calcification in such diseases as hypertension, cancer, and tuberculosis. A single

case, a 65-year-old patient with diabetes, showed 4 plus calcification as compared with an average of 1.8 for that age group. Four patients with cirrhosis of the liver, whose ages ranged from 30 to 69 years, showed a higher degree of calcification by about 1 plus than the average for the corresponding age groups, and in 2 cases of primary carcinoma of the liver there was a quantitatively similar deviation. It thus appears that local obstruction to the flow of blood through the hepatic artery may intensify these age changes.

Renal Artery

Until approximately age 30, with few exceptions, the internal elastic lamella of the renal artery lay in contact with the lining endothelium as in the hepatic artery; and in the micro-incinerated preparations this was again represented by a thin, distinct, wavy line of calcium. On the other hand, the external elastic lamella showed an increase in the number of fibers relatively early, beginning in the second decade of life when the identity of the parent membrane was lost, and usually appearing in the third decade as 6 to 8 parallel, wavy, elastic bands extending well into the adventitia. Correspondingly, by the age of 20 years there was already notable calcification of the external elastic lamella, usually somewhat less than 1 plus. During the third decade also, filaments and granules of material showing the staining qualities of elastic tissue appeared along the inner side of the external elastic bands and extended into the media. Along these extensions foci of calcification began to appear; one case showed areas of 3 plus calcification at this time, but on the average calcium deposition was 1 plus (Figs. 5 and 6).

From the fourth decade on, the intima became progressively thicker due to hyalinization, until in very old specimens it occupied about one-third of the thickness of the wall. The internal elastica lay at the base of the intima, and the parent membrane easily could be identified even in very old specimens. Occasionally there were focal duplications of the internal elastic lamella into the intima; this sometimes was associated with the appearance of gaps in the continuity of the internal elastica. Calcification in such areas was proportional to the degree of elastic multiplication, but rarely exceeded 2 plus in intensity. In the fifth and sixth decades filaments and granules of material with the staining properties of elastic tissue appeared beneath the internal elastic lamella and extended into the media. However, these extensions neither penetrated as deeply nor did they appear as concentrated as those arising adjacent to the external lamella. Intimal plaques were encountered only rarely,

but when they were present there was an intensified duplication of elastic fibrils across the base of the plaque. Some increase in the intensity of granular and filamentous extensions of elastic material into the media was noted also. The latter process is similar to that previously observed in the coronary artery.

As would be expected from the pattern of elastic tissue alteration, calcification was most intense along the external lamella and extended into the media in a pattern duplicating the elastic extensions (Figs. 7 and 8). During the sixth decade approximately one-third of the cases showed many such foci of calcification of 3 plus or greater intensity, and the number of cases showing areas of intense calcification increased with succeeding decades until in the ninth and tenth only a rare case presented less than a 3 plus degree of calcification.

Beginning at about age 50, occasional renal arteries showed a different pattern from that described. Instead of fibrillar and granular extensions moving into the media from the elastic membranes, there was a diffuse arrangement of fine, elongated, elastic filaments through the media, oriented parallel to the internal and external elastic lamellae. Between these filaments there were clumps of elastic material in the form of fine filaments and granules, the quantity of the latter depending upon the age of the vessel. The pattern, therefore, resembled that seen in the aorta, and we have termed this process "aortification." However, the parallel elastic fibrils were thinner and more widely spaced than in the aorta. For the most part, the degree of calcification depended upon the concentration of elastic material between these fibrils, but even when the latter process was not marked, the intensity of calcification was rarely less than 2 plus.

Of the four major categories of disease with which a correlation of the degree of elastic tissue change and intensity of calcium deposition was attempted, hypertension, tuberculosis, and carcinoma did not appear to alter the rate or intensity of these processes except in patients over 80 years of age. In that group, cases with hypertension or carcinoma showed less calcium and a more moderate degree of elastic tissue alteration than the average for that age period. In 3 cases of hypertension the average degree of calcification was 1.2 plus less than for the total age group, while in 4 patients with carcinoma it was 0.5 plus less. There was only one patient with diabetes, age 65, who showed 3.5 plus calcification of the renal artery as compared with an average of 2.6 for the decade 60 to 69. No significance is attached to these findings because of the relatively few cases studied.

Iliac Artery

As in the hepatic and renal arteries described, the inner elastic membrane of the iliac artery lay along the inner surface of the vessel during the first decade of life; it appeared in the micro-incinerated specimen as a finely accentuated white line of calcium. Beneath this there were fairly broad elastic fibers arranged in rows parallel to the inner elastic lamella. The external elastica at first consisted of several wavy bands coursing in the same axis as those noted above, perhaps slightly thicker than those seen in the media. The incinerated specimens showed a cellular distribution of calcium until the latter part of the second decade when granules and fragments of elastic material appeared between the fibers in the media coincidentally with clump formation of calcium in the incinerated sections (Figs. 9 and 10). This deposition of elastic material occurred somewhat more intensely along the outer third of the media usually adjacent to the external lamella. Such clumping was observed only in occasional cases during the latter part of the second decade, but gradually increased in intensity and in area during the next 2 decades, so that by age 39 there was, on the average, a well developed 2 plus calcification.

From the fifth decade on, the internal elastic membrane appeared to split off fine filaments which contributed to the intima which had thickened somewhat by a process of hyalinization; filaments also penetrated into the media. During the sixth decade, in most instances the identity of the inner elastic membrane was lost in a dense mass of elastic material. The external elastic lamella also appeared to split off elastic elements which contributed to the elastic mass in the media. Consequently, calcification occurred first as two bands along the outer and inner thirds of the media, with later extension into the middle third, as the elastic material progressively accumulated in this area. This process began at about age 40, and by the sixth decade there was a well developed 3 plus calcification in most vessels, with elastic material and calcium distributed diffusely through the whole thickness of the wall (Figs. 11 and 12). There usually was less calcification along the inner 20 per cent of the wall which constituted the intima than through the remainder of the vessel, but elastic elements infiltrated the intima and some degree of calcification of this layer was present in almost every vessel after age 60. Succeeding age periods showed only a progressive intensification of this process.

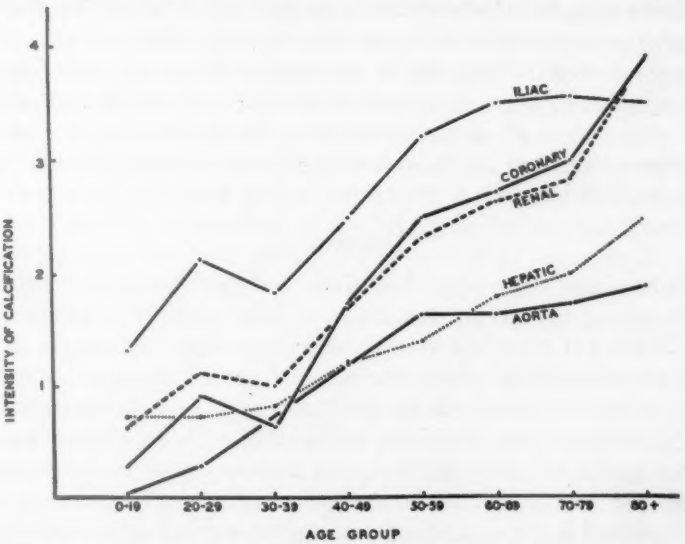
Occasional vessels gave an indication of the mechanism of Mönckeberg's sclerosis. Dense concentrations of granules and filaments of elastic material developed deep in the media of some vessels and these

areas showed intense calcification, some of which was apparent even with routine hematoxylin and eosin stains.

The iliac vessels of hypertensive patients showed no intensification of this process. In patients below age 50 with malignant disease (8 cases), the degree of calcification was less than the general average by approximately 0.5 plus, but in those over 60 (15 cases) the intensity of calcification was higher than the average for the decade 60 to 69 years by about the same average difference. There was no significant deviation from the average in a single case of diabetes and in 12 patients with tuberculosis.

COMPARISON OF THE RATE OF CALCIFICATION IN SEVERAL MAJOR ARTERIES

In Text-Figure 1 we have charted the rate of calcification in the several major arteries studied to date. It should be pointed out, however, that calcification varies from site to site in a given vessel, and the data represent an impression of the average intensity and frequency



Text-Figure 1. A comparison of the rate of calcification of several major human arteries.

of distribution of such foci. The intensity in each instance is based upon arbitrary standards established in the initial studies on the aorta. Quantitative chemical studies will be made at a later date. It is apparent from these curves that the aorta and hepatic arteries calcify relatively slowly compared with the other vessels in the chart. We have

no data on the intensity of elastic tissue fragmentation and granulation in the aorta; a comparison of these changes in the aorta and pulmonary artery are now in progress. However, in the hepatic artery, as noted earlier in this paper, these changes are not as intense as in the coronary, renal, and iliac arteries. Thus, even in relatively old persons, the degree of calcification is rarely more than 2 plus in the hepatic artery and the aorta.

The curves representing the coronary, renal, and iliac arteries have certain similarities. In each instance there is a lower degree of calcification in the fourth than in the third decade, although this difference may not be significant. Furthermore, all three vessels show a relatively rapid rise in calcium content between the fourth and seventh decades; calcification in the iliac artery then tends to level off, whereas in the coronary and renal arteries it continues to increase through succeeding age periods. The curves representing the rates of calcification of the coronary and renal arteries are almost superimposed upon each other; the differences at corresponding age periods are probably not significant.

These data do not show that in all age periods in which there is a notable average degree of calcification there are cases showing a low degree of elastic change and a low degree of calcification. Even in patients over 80 years, occasional vessels are encountered showing only a 1 plus degree of calcium deposition. Such data suggest that the maximum degree of calcification is approximated in the third or fourth decades, following which the curve tends to level off.

Discussion

It has been demonstrated in these and previous studies that the basic ageing process in the major arteries of man is primarily a calcification of the media with extension of this process into the intima and adventitia. The calcification appears to be intimately associated with certain alterations in the physical character and in the pattern of distribution of the elastic tissue, these changes in the elastic elements being an integral part of the ageing process. Thus, in the coronary artery the greater part of the elastic fragments and granules seem to be deposited in the area about the internal elastic lamella, and this is the site in which calcium deposition is most extensive[5]; in the renal artery these changes are predominant about the external lamella, and the most extensive calcification occurs in this location. The hepatic artery develops an intermediate pattern with both lamellae apparently contributing to the ageing process, although it is more prominent in

the region of the internal elastic band; furthermore, the rate and intensity are considerably diminished. In the iliac artery the ageing process produces a distribution of elastic elements in the media resembling that found in the aorta, but the process is most intense in the areas adjacent to the elastic lamellae, and in these locations calcification also is most intense. A similar process which we have termed "aortification" is observed occasionally in the renal and coronary arteries, but it is a relatively rare occurrence in these vessels, and appears to have no relation to any of the several diseases for which a statistical correlation was attempted.

It has been shown also that the intensity and rate of calcium deposition are directly proportional to the intensity and rate of these elastic tissue changes. Thus in the iliac artery there is a more intense deposition of filaments, fragments, and granules of elastic staining material than in the other arteries in corresponding age groups. The intensity and rate of deposition of elastic material and calcium are about the same in the coronary and renal arteries at corresponding ages, and less in the hepatic artery. No histologic studies of elastic tissue changes in the aorta have been reported because of the difficulty in estimating the quantity of these altered forms of elastic material in a vessel containing an abundance of elastic tissue even in very young patients. Instead, the aorta is being studied by analytical chemical technics.

No significant intensification or retardation of these processes has been noted in relation to such diseases as hypertension, diabetes, malignant tumors, or tuberculosis in the relatively few cases included in this report. It has been noted, however, that in diseases such as cirrhosis or carcinoma of the liver, which may produce obstruction of the hepatic artery, there is an intensification of the ageing processes in this vessel.

We have made the point in our introduction that these are ageing processes common to many tissues, and not distinctive of blood vessels; as such they represent manifestations of fundamental age changes in tissues. The problems connected with the ageing of blood vessels, therefore, are not basically different from those of other tissues. Lansing[6] has demonstrated that an increase in tissue calcium is a fundamental part of the ageing process in lower forms of animal life, as well as in mammals. Furthermore, the elastic tissue changes do not represent a phenomenon specifically encountered in the vascular system. Unna[7] described similar changes in the skin in aged persons, and Weidman[8] has studied the phenomenon of "elastosis senilis," which he considered

a physiologic accompaniment of cutaneous senescence. Weidman was of the opinion that it should cause no surprise if calcification is found in the cutaneous deposits of elastic tissue, but this process apparently has not been studied in the skin. Calcification is, however, an accompaniment of pseudoxanthoma elastica, a skin condition in which an increase in elastic elements occurs. Bittrolff[9] also has observed calcification in association with changes in elastic elements in the lungs similar to those which we have described in arteries. Wells[1] stated that similar processes occur in the elastic cartilage of certain joints and in this structure the changes in elastic elements and the increase in calcium are accompanied by the appearance of cholesterol.

The association of calcium deposition with changes in the elastic elements in human vessels likewise has been observed previously. Ravault[10] suggested that calcification of the media of the aorta is primarily associated with the elastic elements, since in the early stages calcium is deposited between muscle fibers rather than inside the muscle cell. Similarly, Ku[11] has noted a parallelism between the increase in elastic tissue and the ash in several coronary arteries, and Zinkant[12] has observed a similar association in micro-incineration studies of human uterine arteries. We have pointed out previously that the loss of elasticity of the aorta parallels the rate of increase of calcium in the media.[2]

This association of elastic tissue changes and calcium deposition is not limited to human arteries. It has been described by Fox[13] in several species of animals, and is perhaps most pronounced in the rabbit, cow, and bird. In the rabbit the intimal changes seem to be due to a fibrillar thickening in which fine elastic fibrils participate and to which a small deposit of sudan-staining material and narrow strips of calcium may be added. In the muscle of the media there are clear calcium plates both near and removed from the lumen. These appear to be nearest the elastic fibers, and, when in the intima, they occupy a position just below the internal elastic lamella. Lucien and Parisot[14] maintained that rabbit arteriosclerosis is comparable to the human disease and that severe spontaneous cases are the same as experimental cases. Ophüls[15] likewise was of the opinion that severe spontaneous rabbit arteriosclerosis is quite similar to the natural human disease. Jaffé[16] emphasized the occurrence of calcification of the media in the rabbit aorta and scarcely credited the appearance of atheroma.

Farkas and Fasal[17] have noted that calcification of arterial walls becomes visible before the formation of intimal plaques. As in some of

our specimens, they encountered plaques beginning with the 20th year, but believed that this change has no causal relationship to arteriosclerosis. We also have presented evidence[2] to show that in man medial calcification precedes the formation of intimal plaques. This may be the case likewise in spontaneous arteriosclerosis in the animals commonly used in the experimental production of arteriosclerotic lesions, namely, rabbits and birds. It is likely, in many instances, that if sufficiently old animals are used, the ease with which atheromatous plaques are produced by cholesterol administration is dependent upon the state of development of the spontaneous process of elastic calcification. There is evidence to substantiate such an opinion. Anitschkow[18] and also Harrison[19] have shown that the production of medial defects in the rabbit aorta influences the deposition of intimal lipids, and Wilens[20] has immobilized segments of vessels by placing silver cuffs about the femoral and carotid arteries of rabbits, a procedure which results in adventitial thickening and fibrosis as well as a thinning of the media and condensation and fragmentation of elastic fibers. This latter process leads to a selective localization of lipids in the intima of arteries at the region of the cuffs when cholesterol is subsequently administered. It has been observed by Schmidtmann and Hüttich[21] that adrenalin injected into rabbits produces medial necrosis and facilitates the deposition of administered cholesterol. It is of pertinent interest that in unpublished studies we have not been able to demonstrate the elastic tissue changes and calcium deposition in several major arteries in the mouse, and that this species is notoriously resistant to experimental cholesterol atheromatosis.

Wells[1] recognized that the alterations that constitute arteriosclerosis depend chiefly on the colloidal properties of elastin, and that the changes in the elastic elements of arteries are in striking agreement with the well known behavior of ageing colloids in general. Among these alterations with age he noted (1) a reduced capacity to bind water, (2) a decrease in elasticity and flexibility, (3) a decrease in permeability, and finally (4) a tendency for the gel to be transformed into a granular state with a marked decrease in the colloidal properties. Bürger and Schlomka[22] looked upon the loss of water as the primary process, with a resulting decrease in the capacity of the colloids to hold soluble constituents such as cholesterol and calcium in solution.

As to the granular changes in the elastic elements which have been described here, Wells[1] attributed this also to a loss of water, but recognized the likelihood that some chemical change also had occurred

as indicated by the increased tendency to bind basic stains, often associated with a decreased affinity for the usual elastic stains. Little is known of the properties of elastin, or even of its origin. Cytologists believe that there are no specific cells that produce elastin; elastin appears to be laid down by fibroblasts of the same type as those which produce collagen. Despite this lack of knowledge, it is generally assumed that the granular material is a degeneration product of elastin. If this were so, the result in old persons would be a decrease in elastic material. Such a conclusion cannot be reached from the present studies; the amount of granular and filamentous elements far exceeds that which one would expect to be derived from the intact elastic lamellae. It must be assumed that either new elastic material is laid down to reconstitute the inner and outer lamellae, and that these then undergo degeneration, or that the granular and filamentous elements are laid down as such in progressively increasing amounts with increasing age. In favor of the former view are the recent observations of Gross[23] that normal elastic tissue contains two distinct chemical and morphologic components, namely, threads and an amorphous binding substance, which are associated to form the elastic fiber. In this connection it is interesting that there is a tendency of the threads to aggregate on the acid side and to fray into finer threads at higher pH. It would thus appear that a higher pH in the vessel wall would facilitate not only fraying and breakdown of elastic elements, but also the precipitation of calcium salts. Against this hypothesis is the fact that the identity of the parent elastic membranes is frequently lost with the marked increase in filaments and granules. It is possible, however, that in old arteries intact elastic fibers break down into their component elements as rapidly as they are formed.

Likewise our knowledge of the chemical constitution of elastic tissue is scant. It is apparently a protein which Wells[1] considered of an unusual sort in that it appears to be about half glycine and leucine. Most of the studies to date have been rather crude. Its affinity for orcein may be taken as indirect evidence that it probably contains a carbohydrate moiety, and this may be of importance in that Hass[24] has observed that in ageing of costal cartilage the polysaccharide moiety decreases as calcium is deposited. This may explain the loss of affinity for orcein of the filamentous and granular material as it progressively calcifies. Despite this meager state of our knowledge of elastic tissue, the material constituting this component of arterial walls is referred to as a chemical unit called elastin. Weidman[8] has postulated that in ageing processes in the skin, collagenic fibers be-

come impregnated with a substance which he calls pre-elacin, and in this way he accounts for the increase in elastic elements. Unna[7] showed that the elastic material in skin changes from an acidophilic to a basophilic state in the course of ageing, and he referred to this altered form as elacin, thereby indicating a chemical transformation to a new form of elastic material. Until more is known of the chemical nature of the intact elastic membrane as well as of the granular and filamentous forms, we would prefer to term the latter elements elastoid, thus indicating that they retain, in large part, the specific staining properties of elastic tissue, but have an altered physical form which has resulted in a loss of elastic efficiency.

Finally, it should be pointed out that the process of elastoid deposition and calcification in arteries is not uniform but, like the formation of atheromata, it is focal in character; neither is it uniform in any group. As we have pointed out, there are occasional young vessels showing active elastic change and calcification, and conversely, there are relatively old vessels in which these alterations are minimal. The pattern of these ageing processes appears to be determined in the third or fourth decade of life. Like Thoma and Kaefer,[25] Klotz,[26] Beitzke,[27] and Wells,[1] we believe that arteriosclerosis consists primarily of the progression of chemical and physical changes in elastic tissue which reduce the resiliency of the arterial wall and result in dilatation of the vessel. The subsequent changes, such as the formation of atheromata, seem to be secondary to these processes in the media.

These observations indicate the importance of studies of the metabolism of the arterial wall and its component parts along the lines recently reported by Briggs, Chernick, and Chaikoff[28] and by Chernick, Srere, and Chaikoff.[29] Such experiments may serve to determine whether there is an intramural origin of lipids responsible for the atheromata rather than the time-honored concept of diffusion of these substances from the plasma through the endothelial lining. Studies of this type may also yield information concerning the release of substances having an injurious effect upon the muscular component of the arterial wall as recently suggested by Szent-Györgyi.[30]

Summary

The hepatic, renal, and iliac arteries of approximately 140 human cases have been studied by means of routine hematoxylin and eosin and elastic tissue preparations, and by micro-incineration; a comparison has been made of the age changes in these vessels and those in the aorta and coronary artery as previously reported.

The present investigations reaffirm our previous conclusion that the basic age change in the major arteries of man is primarily calcification of the media. These studies show that the calcification is intimately associated with alterations in the physical character and pattern of distribution of the elastic tissue, which are described; the intensity and rate of calcium deposition is directly proportional to the intensity and rate of the elastic tissue changes.

It has been demonstrated further that the location of these processes within the wall is different in different vessels, as is also the rate of calcification. The iliac artery calcifies most rapidly, followed by the renal and coronary arteries which show an almost identical rate of calcium deposition. The aorta and hepatic artery calcify relatively slowly.

An attempt has been made to correlate certain disease processes with the intensity of the age changes. In general, the number of cases of a specific disease in a given age group is too small to warrant any conclusion, but there appears to be an intensification of the ageing processes in the hepatic artery of individuals with diseases such as cirrhosis and carcinoma of the liver which may obstruct the hepatic blood flow.

These observations yield additional information on the life history of elastic tissue. The term elastoid is suggested for the fragments and granules of elastic material which increase in the ageing process of arteries, pending further knowledge of their physical and chemical characteristics.

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DESCRIPTION OF PLATES

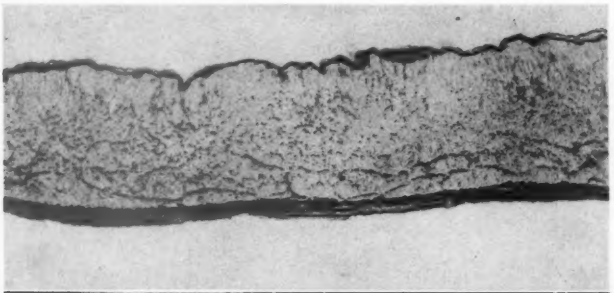
PLATE 141

FIG. 1. Section of the hepatic artery of a male, 19 years old, showing beginning extension into the media of elastic fragments and granules from both elastic lamellae. Resorcin-fuchsin stain. × 90.

FIG. 2. From the same case as Figure 1. Micro-incinerated section. The calcium line along the inner surface of the vessel corresponds to the inner elastic lamella. × 90.

FIG. 3. Section of the hepatic artery of a male, 65 years old. Of note are the elastic extensions into the intima from the inner elastica and the extensions into the media from both elastic layers. The external elastica has thickened also. Resorcin-fuchsin stain. × 90.

FIG. 4. From the same case as Figure 3. Micro-incinerated section, showing calcification of the inner elastica and extensions of elastic tissue into the intima. In this case there is also calcification of the external lamella. Moderate calcification of elastic extensions into the media may be seen. × 90.



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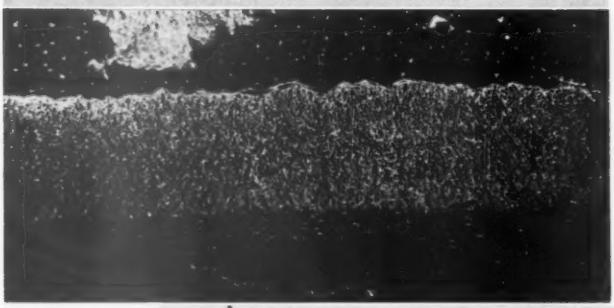

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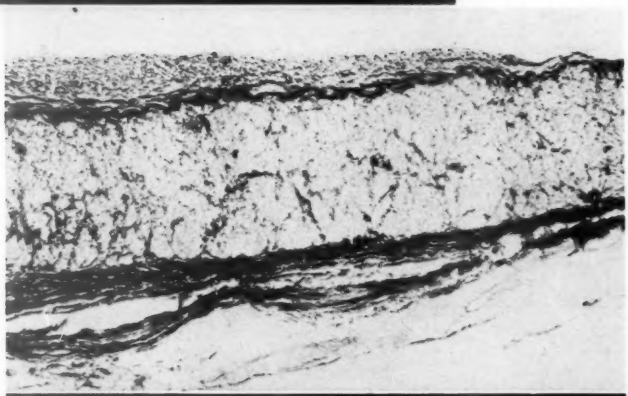

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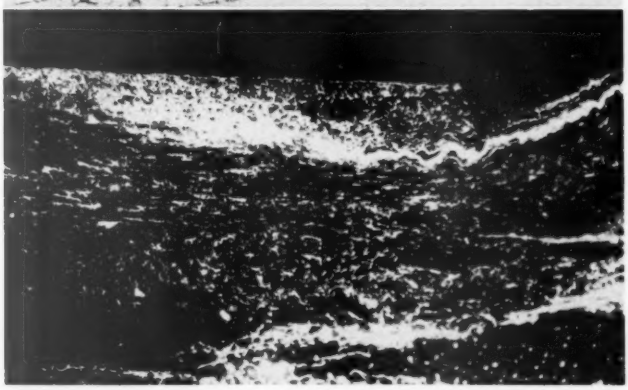

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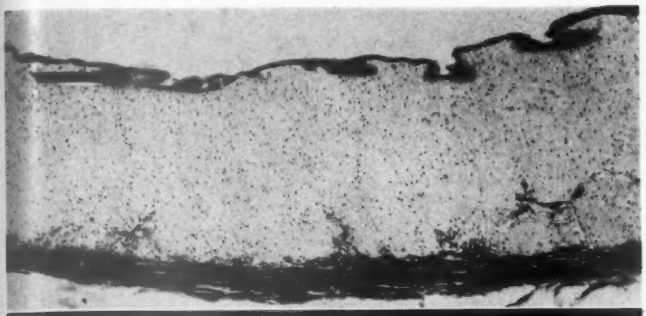
PLATE 142

FIG. 5. Section of the renal artery of a male, 19 years of age, showing a relatively thick external elastic lamella and beginning extension of elastic elements into the outer media. Resorcin-fuchsin stain. × 90.

FIG. 6. From the same case as Figure 5. Micro-incinerated section. The fine calcium line corresponds to the inner elastic lamella. × 90.

FIG. 7. Section of the renal artery of a male, 62 years old. Marked elastic proliferation of the external lamella and numerous elastic extensions into the media may be seen. The inner elastic bundle remains as a single band in most areas. Resorcin-fuchsin stain. × 90.

FIG. 8. From the same case as Figure 7. Micro-incinerated section. Of note is the intense calcification of elastic elements in the media and of the external elastic lamella. The inner elastic lamella can still be identified as a fine line of calcium. × 90.



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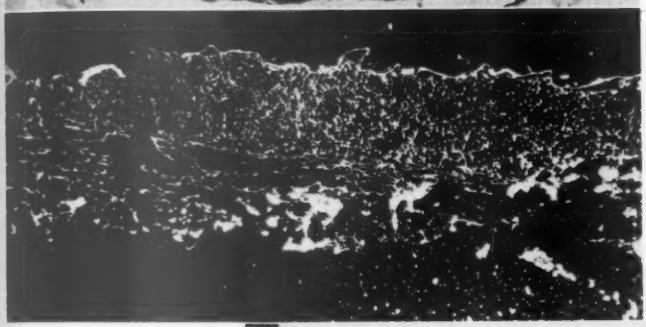

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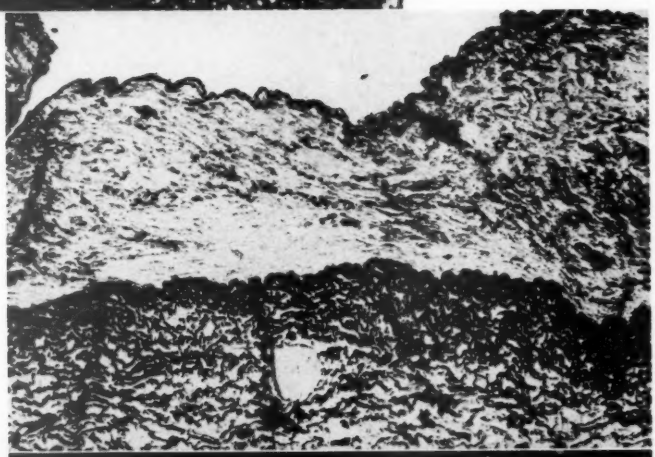

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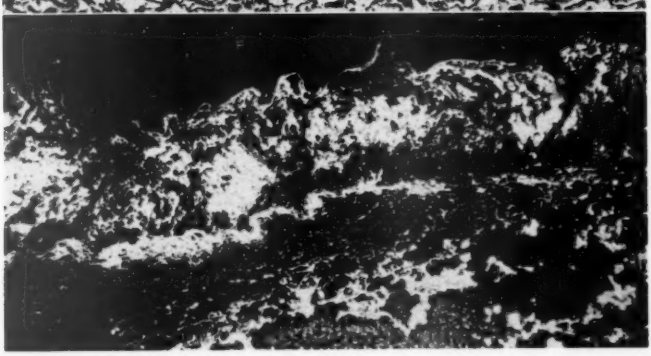

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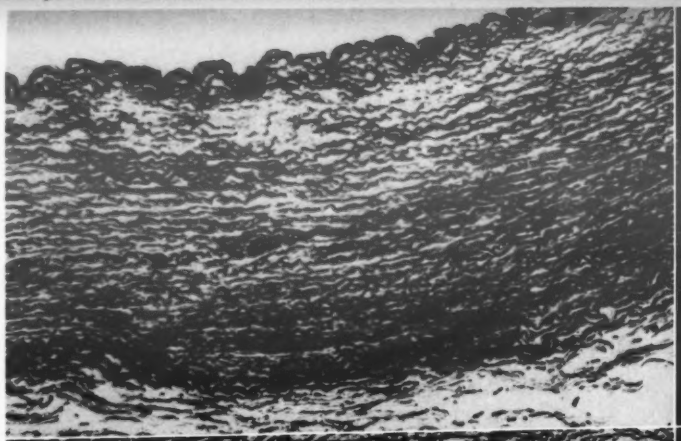
PLATE 143

FIG. 9. Section of the iliac artery of a male, 18 years old, showing a distinct inner elastic lamella, but a relatively indistinct external elastic band. Many plump elastic fibers are present in the media, but discontinuities are beginning to develop. Resorcin-fuchsin stain. × 90.

FIG. 10. From the same case as Figure 9. Micro-incinerated section, showing slight intensification of calcium along the inner surface of the vessel and slight clumping in some areas of the media. × 90.

FIG. 11. Section of the iliac artery of a male, 58 years of age, showing a decrease in thickness of elastic fibers and increase in granular elements between fibers. Loss of identity of the elastic lamellae may be noted. Resorcin-fuchsin stain. × 90.

FIG. 12. From the same case as Figure 11. Micro-incinerated section. There is a diffuse distribution of calcium with loss of calcium lines by which elastic lamellae can be identified. × 90.



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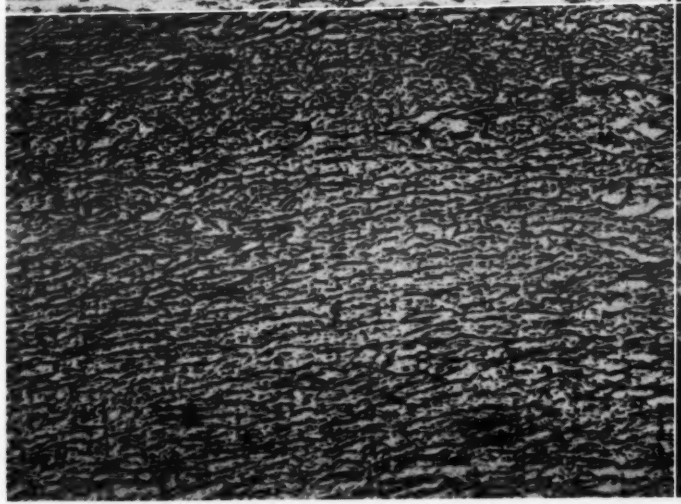

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Blumenthal, Lansing, and Gray

Elastic Tissue and Calcium in Arteriosclerosis

LOCAL CELLULAR RESPONSES IN EXPERIMENTAL HYPERSENSITIVITY *

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The present studies were undertaken in an attempt to clarify the conflicting statements concerning the nature of the inflammatory response to antigens in the hypersensitive animal, more particularly in bacterial allergy of the delayed or tuberculin type. Dienes and Mallory [1] have indicated the significant rôle of the monocyte in local skin reactions of this type. They also have claimed that sensitivity of the anaphylactic type, tested 3 to 6 days after sensitization, exhibits a predominantly monocytic infiltration in all stages of development of the local reaction. In conflict with these conclusions are the studies of Follis,[2] who found no special cellular response characteristic of bacterial allergy of the delayed type. The conclusions of LaPorte [3] are, in our opinion, too ambiguous to lend support to either of the above observations. The problem has assumed increasing significance since the observation of Landsteiner and Chase [4] concerning the passive transfer of sensitivity of the tuberculin type by means of leukocytes from the sensitive animal. The type of cell involved here has not been determined with certainty.

Due to the difficulties in interpretation of cell types in histologic preparations of skin, parallel studies were undertaken using the response of the peritoneum to the specific antigen.

Materials and Methods

Guinea-pigs weighing approximately 300 gm. and rabbits weighing approximately 2,500 gm. were used. Sensitivity was induced by either egg albumin three times crystallized by the method of Kekwick and Cannan [5] and standardized by nitrogen determination, or BCG.† All intracutaneous injections were made on the flank in a volume of 0.05 cc. with a 27 gauge needle. The test antigen in the BCG sensitized animals was either the purified protein derivative (PPD) of Seibert‡ or old tuberculin § (OT) as indicated.

Initial attempts to use the peritoneum of the guinea-pig were abandoned because of the large numbers of lymphocytes and monocytes in

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† Kindly furnished by Dr. S. R. Rosenthal, Tice Laboratory, Chicago, Ill.

‡ Furnished through the courtesy of Sharp & Dohme, Inc., Philadelphia, Pa.

§ Eli Lilly & Co., Indianapolis, Ind.

the untreated peritoneal cavities of all 8 animals examined. Total counts on the peritoneal fluids ranged from 8,000 to 52,000 per cmm., with an average of 24,000. The average percentage of monocytes and lymphocytes was 88. In the rabbit this situation was not encountered. In 6 normal rabbits the average total white blood cell count on peritoneal fluid was 700, with a range from 50 to 1,400 per cmm. These cells were predominantly mononuclear.

Peritoneal fluid was obtained immediately after killing the animals with intravenous nembutal. Differential counts on the peritoneal exudates were made by the supravital technic described by Sabin[6]; total white blood cell counts were taken from two or three different sites in the peritoneal cavity and averaged. Histologic sections of the site of intracutaneous injection and of the omentum were prepared and stained with hematoxylin and eosin.

The differential percentages of inflammatory cells in sections of skin as given in Tables I and II represent estimates derived from several widely different sites in the area of reaction. In view of the variation in different areas of each lesion it seemed that actual cell counts rather than estimates would not afford additional accuracy. The fibrocytes in many instances resembled monocytes or plasma cells and offered considerable difficulty in differentiation. The histologic findings were classified on the following basis:

o	Negative
±	Occasional inflammatory cells only
+	Inflammatory cells scattered diffusely through the corium
++	As above, but with focal accumulation of inflammatory cells and slight edema
+++	Continuous inflammatory lesion involving all layers of the skin, with marked edema

Experimental Findings

Cellular Response in Early Anaphylactic Sensitivity

Guinea-pigs were sensitized by the intra-abdominal injection of crystalline egg albumin either alone or adsorbed on alumina[7] and tested 4 to 6 days later, before anaphylactic sensitization would be expected to become well established. Table I indicates in each case the amount of antigen used, the duration of skin reaction which is the time elapsing between the intracutaneous injection and the sacrifice of the animal, and the results as determined by examination of histologic sections.

A comparison of the reactions of the normal and sensitized animals to egg albumin showed no essential difference, and in only one of 18 reactions—guinea-pig 23 after 6 hours—was the monocyte the predominant

cell. Furthermore, comparison of the reaction to normal saline solution and the reaction to egg albumin, in both normal and sensitized animals, revealed a slight, predominantly polymorphonuclear, leukocytic response which did not differ significantly in any of the groups tested. The marked variation of response within each group of similarly treated animals was noteworthy.

Early Cellular Changes in Established Bacterial Allergy

Rabbits received BCG by subcutaneous, intrathoracic, or intravenous inoculation and were tested after intervals of 47 to 69 days. In most cases sensitivity was proved by intracutaneous injection of either 0.5 to 1.0 mg. of PPD or 1 mg. of OT in 0.05 cc. before the final tests were performed.

Table II shows the amounts of BCG used for sensitization, the route of injection, the time elapsing before testing, the duration of the dermal and peritoneal reactions, and the results of histologic examination of the skin and peritoneum.

The data in Table II clearly indicate that the initial inflammatory response to tuberculin in the skin and peritoneum of the rabbit involved predominantly neutrophilic granulocytes. In a few guinea-pigs, not shown in Table II, similar results were obtained in the skin although for the reason mentioned above it was not possible to study the peritoneal reaction. The increase in monocytes after 24 hours conformed to the usual pattern of inflammation.

Discussion

In a large number of allergic reactions in the skin and peritoneum of sensitized rabbits and guinea-pigs nothing has been found to suggest that in the early stages of these reactions the monocyte was exclusively or even predominantly involved, although it was present in variable numbers. It is our opinion that early allergic inflammation does not differ from that caused by other types of injury of a similar intensity. The fact that the peritoneal reactions, which could be studied with greater accuracy, conformed in their cellular make-up to those occurring in the skin supports this conclusion.

Because of the remote possibility that subcutaneous injection with BCG might produce an exclusively dermal sensitivity, the intrathoracic and intravenous routes also were employed in the studies on the peritoneal reaction. No differences were encountered, however, when animals injected by these means were tested.

Although the findings of Dienes and Mallory [1] have not been confirmed with respect to the cytology of the reaction, it is not implied that

TABLE I

Skin Reactions in Sensitized Guinea-Pigs

Guinea-pig no.	Sensitization procedure and duration		Duration of reaction‡	Skin test material					
				0.1 mg. EA*			Saline solution		
				Degree of reaction	Polymorphonuclear cells	Mononuclear cells	Degree of reaction	Polymorphonuclear cells	Mononuclear cells
		days	*hours*		*per cent*	*per cent*		*per cent*	*per cent*
23	10 mg. EA* IA†	4	1	+	95	5	±	40	60
24	10 mg. EA IA	4	1	+	50	50	+	80	20
25	10 mg. EA IA	20	1	±	50	50	±	50	50
26	10 mg. EA IA	20	1	+	60	40	0		
D	Not sensitized		1	+	60	40	+	60	40
H	Not sensitized		1	±	50	50	0		
23	10 mg. EA IA	4	6	++	30	70	±	50	50
24	10 mg. EA IA	4	6	++	80	20	±	50	50
25	10 mg. EA IA	20	6	+	70	30	+	70	30
26	10 mg. EA IA	20	6	+	60	40	+	70	30
D	Not sensitized		6	+	60	40	+	60	40
H	Not sensitized		6	±	40	60	+	70	30
23	10 mg. EA IA	4	24	++	70	30	±	50	50
24	10 mg. EA IA	4	24	+	50	50	±	50	50
25	10 mg. EA IA	20	24	+	60	40	+	80	20
26	10 mg. EA IA	20	24	+	50	50	+	70	30
D	Not sensitized		24	±	50	50	±	50	50
H	Not sensitized		24	+	80	20	0		
101	50 mg. EA and alumina IA	4	6	+	60	40	0		
102	50 mg. EA and alumina IA	4	6	+	90	10	±	60	40
103	50 mg. EA and alumina IA	4	6	+	70	20	+	50	50
104	50 mg. EA and alumina IA	4	6	+	70	30	+	80	20

105	50 mg. EA and alumina IA 6	6	+	80	20	+	60	40
106	50 mg. EA and alumina IA 6	6	+	80	20	±	60	40
107	50 mg. EA and alumina IA 6	6	+	80	20	+	90	10
108	50 mg. EA and alumina IA 6	6	+	80	20	+	90	10
AA	Not sensitized	6	+	70	30	0		
BB	Not sensitized	6	+	80	20	±	60	40
CC	Not sensitized	6	+	70	30	±	60	40
DD	Not sensitized	6	+	80	20	0		
101	50 mg. EA and alumina IA 4	24	+	70	30	0		
102	50 mg. EA and alumina IA 4	24	+	60	40	0		
103	50 mg. EA and alumina IA 4	24	+	80	20	0		
104	50 mg. EA and alumina IA 4	24	+	60	40	0		
105	50 mg. EA and alumina IA 6	24	++	60	40	±	60	40
106	50 mg. EA and alumina IA 6	24	+	80	20	0		
107	50 mg. EA and alumina IA 6	24	++	70	30	+	50	50
108	50 mg. EA and alumina IA 6	24	++	80	20	+	30	70
AA	Not sensitized	24	+	50	50	+	50	50
BB	Not sensitized	24	+	60	40	+	50	50
CC	Not sensitized	24	+	80	20	++	80	20
DD	Not sensitized	24	+	90	10	±	70	30

* Crystalline egg albumin.

† Intra-abdominal.

‡ Duration of reaction is the time elapsing between the test injection and the removal of the skin for fixation.

TABLE II

Dermal and Peritoneal Reactions in Sensitized Rabbits

Rabbit no.	Sensitization procedure, route, and duration	Skin reaction				Peritoneal exudate 1 mg. PPD in 1 cc. saline solution				
		Duration in hours*	Degree of reaction	Polymorphonuclear cells	Mononuclear cells	Duration in hours*	Total white blood cells	Polymorphonuclear cells	Monocytes	Lymphocytes
	days			*per cent*	*per cent*			*per cent*	*per cent*	*per cent*
				1 mg. PPD						
37	30 mg. BCG SC† 47	6	++	90	10	48	3,400	3	94	3
38	30 mg. BCG SC 47	6	++	90	10	24	10,100	82	15	3
39	30 mg. BCG SC 47	6	+++	90	10	6	10,800	93	7	
40	30 mg. BCG SC 47	6	++	90	10	6	13,000	87	13	
M	Not sensitized	6	+	90	10	48	3,750	10	85	5
N	Not sensitized	6	±	60	40	24	1,100	90	8	2
O	Not sensitized	6	±	80	20	6	30,000	96	4	
P	Not sensitized	6	±	80	20	6	41,000	96	3	1
37	30 mg. BCG SC 47	48	++	60	40					
38	30 mg. BCG SC 47	48	++	60	40					
39	30 mg. BCG SC 47	48	++	70	30					
40	30 mg. BCG SC 47	48	++	80	20					
M	Not sensitized	48	0							
N	Not sensitized	48	0							
O	Not sensitized	48	±	50	50					
P	Not sensitized	48	±	40	60					
				0.5 mg. PPD						
35	2 injections 30 mg. BCG SC 69	6	+++	90	10	6	107,000	100		
L	Not sensitized	6	++	90	10	6	66,000	94	4	2
34	2 injections 30 mg. BCG SC 69	24	++	60	40	24	18,000	82	16	2
K	Not sensitized	24	+	90	10	24	8,000	60	40	

33	2 injections 30 mg. BCG SC 69	48	+	50	50	48	21,600	12	84	4
5	Not sensitized	48	0			48	18,200	36	60	4
				OT 1:50						
13	15 mg. BCG IT‡ 60	6	++	30	70	6	46,000	100		
14	15 mg. BCG IT 60	6	+	70	30	6	24,000	96		4
15	15 mg. BCG IT 60	6	+	80	20	6	15,000	97		3
2	15 mg. BCG IV§ 60	6	++	90	10	6	17,000	99	1	
9	15 mg. BCG IV 60	6	+	90	10	6	37,000	98	1	1
10	15 mg. BCG IV 60	6	+	80	20	6	7,000	98	2	
4	Not sensitized	6	±	90	10	6	17,000	99	1	
5	Not sensitized	6	+	90	10	6	32,000	98	1	
6	Not sensitized	6	+	90	10	6		98	2	
7	Not sensitized	6	±	80	20	6	13,000	100		
11	15 mg. BCG IT 60	24	++	70	30	24	12,000	45	16	39
12	15 mg. BCG IT 60	24	±	60	40	24	22,000	100		
8	15 mg. BCG IV 60	24	++	40	60	24	29,000	7	73	20
1	Not sensitized	24	+	80	20	24	4,750	90	9	1
3	Not sensitized	24	+	60	40	24	1,250	93	5	2

* Duration of dermal or peritoneal reaction is the time elapsing between the test injection and removal of skin or peritoneal fluid for examination.

† Subcutaneous.

‡ Intrathoracic.

§ Intravenous.

response of the delayed or tuberculin type fails to occur in incipient anaphylactic sensitization.

SUMMARY

Guinea-pigs which were tested early in the development of sensitivity to egg albumin failed to exhibit a predominantly monocytic response.

Rabbits sensitized with BCG and tested with PPD or OT in the skin and peritoneal cavity developed an inflammatory response in which the neutrophilic granulocyte dominated the picture in the early stages.

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UNUSUAL GLOMERULONEPHRITIS IN YOUNG CHILDREN, PROBABLY RADIATION NEPHRITIS

Report of Three Cases *

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Roentgen rays of high voltage are widely used in the treatment of renal neoplasms. Dosage and mode of application are well established, and within the accepted limits this form of therapy is generally regarded as safe. The recent literature contains no reports of impairment in renal function or of lesions in the kidney attributed to radiation. Earlier workers were able to produce renal lesions in experimental animals with roentgen rays, but the kidneys are considered to be only moderately susceptible to radiation and do not fall into the category of radiosensitive organs.[1]

In view of these facts we were somewhat hesitant in reporting the development of an unusual nephritis with fatal outcome following exposure to radiation in conventional therapeutic dosage. The repetition of the same sequence of events in 3 cases, however, made coincidence seem unlikely. The similarities in the age and the clinical course of the 3 patients, in the evidence of normal renal function and structure prior to radiation, in the comparable time interval between exposure and the onset of renal failure, and in the identity of the pathologic changes in the kidneys point to a causal relationship.

We therefore present our findings in order to call attention to the possible dangers of conventional radiation therapy over the renal area of young patients and to stimulate further investigation which may support or disprove our hypothesis.

Report of Cases

Case 1

B. E., a 4-year-old white girl, was first admitted because of abdominal pain and enlargement, and hematuria in August, 1946. A large abdominal mass was palpable on the left side. Intravenous pyelograms showed good function and normal contour of the right kidney, while the left kidney could not be visualized. The urine had a specific gravity of 1021 and contained 2 plus albumin and moderate numbers of leukocytes. The blood urea nitrogen was 13 mg. per cent.

At operation, an embryoma of the left kidney was found, and a left nephrectomy was performed. Histologic examination confirmed the diagnosis of embryoma, and showed normal structure of the kidney proper. The results of postoperative urinalyses were normal.

* Received for publication, December 9, 1949.

Between September, 1946, and January, 1947, the patient was given three courses of radiation therapy. Each course consisted of ten daily exposures alternating between the lumbar region and the abdomen on successive days, each of 15 minutes with a portal of 15 by 15 cm., at 50 cm. skin distance, at 200 kv., and 20 ma. and through a 1.5 mm. Cu, and 1 mm. Al filter. The daily dosage was 195 r. as measured in air. The combined dosage for each course was 1950 r. The total dosage given in the three series of treatments was 5850 r.

Following the last course, the patient had anorexia and lost weight. She was readmitted in March, 1947, with a clinical picture of renal failure, hypertension, and severe anemia. At that time there was constant albuminuria, slight hematuria, and azotemia. She died 2 months after the last course of radiation therapy.

Post-mortem Examination. At autopsy, no trace of metastatic or recurrent tumor was found. The region of the left kidney was transformed into firm scar tissue surrounded by fat. The right kidney was slightly enlarged, weighing 72 gm., and was firm, pale, and adherent to the capsule. On cut surface, the cortex had a mottled irregular appearance. The striations were lost, and brownish ill defined areas suggested focal hemorrhages. The medulla was pale and showed distinct striations. The pelvis and ureters were not unusual.

The heart was enlarged, weighing 136 gm., and the left ventricle was hypertrophied. There was pulmonary edema and congestion, and marked edema of the stomach, small and large intestine, and the mucosa of the bladder. Small amounts of fluid were present in the peritoneal, pleural, and pericardial cavities.

The microscopic appearance of the kidney was striking and contrasted sharply with that of the other kidney removed surgically 7 months earlier. The structural pattern was distorted by loss or fibrous replacement of many glomeruli; enlargement, hemorrhage, or necrosis of others; dilatation, atrophy, necrosis, or focal disappearance of tubules; and marked increase in interstitial connective tissue (Fig. 1). The changes were not uniform, but widely disseminated.

It was estimated that less than 20 per cent of the glomeruli appeared normal in size and structure. The most frequent lesion, involving approximately 60 per cent of the glomeruli, consisted in damage to the basement membrane and endothelium of the glomerular capillaries with obstruction of the loops. Such glomeruli were moderately enlarged, and had bulky, bloodless loops which were sometimes fused but more often were unusually distinct. The loops were obstructed by a felt-like, fibrillar, granular, or amorphous material derived from the irregularly thickened spongy basement membranes (Figs. 2, 3, and 4). Within this material, as well as in the membranes themselves, small, irregular globules of fat often were demonstrable. The obstructing fibrillar and granular material stained pink with eosin, dirty bluish with azocarmine,

light green with tetrachrome stain, and muddy brown with van Gieson's stain. The endothelial cells were decreased in number or absent. The residual lumina often contained large, pale, foamy, fat-filled macrophages (Fig. 5).

In contrast to the destruction of endothelium and basement membrane, the visceral and parietal epithelium, as a rule, was well preserved, although the cells sometimes were swollen. In some glomeruli fibrinoid necrosis of the tufts (Fig. 6) and fibrinoid thrombi within the lumina were noted. These findings were similar to changes involving the afferent arterioles which will be described separately.

While the obstruction resulting from these changes rendered most of the capillary loops bloodless, occasional loops and sometimes entire tufts were distended with red blood cells. As a rule, however, this was due not to filling of the lumina but rather to hemorrhage into the felt-like masses and the splintered basement membranes (Fig. 7). The erythrocytes were contained within such loops and had not entered the capsular spaces.

Apart from the changes involving the capillary tufts, there were apparently independent alterations in the parietal layer of Bowman's capsule. The basement membrane of this structure often was markedly thickened in such a manner that bulbous projections of hyalin-like material were formed which seemed to constrict the glomerular hilus (Fig. 8). Other glomeruli showed localized thickening (Fig. 9), or enormous cap-like masses of fibrous or hyaline material eccentrically placed opposite the hilus. These fibrous "caps" also seemed derived from the basement membrane, but often merged imperceptibly with the adjacent connective tissue stroma (Fig. 10). The masses encroached on the capsular spaces, but the parietal epithelium, although invaginated, remained intact, so that adhesions or crescents were not formed, and the visceral and parietal layers remained distinct (Fig. 11). In such glomeruli, however, shrinking and compression of the tufts, and replacement of the capillaries by hyaline material was evident. The final stage of this process was the complete disappearance of the tufts leaving a scar of eccentric fibrous and hyaline material with irregular convoluted outlines indicating the original position of the parietal basement membrane (Fig. 12). Approximately 20 per cent of the glomeruli had been destroyed in this manner.

There seemed to be good correlation between the glomerular lesions and the changes observed in the tubules. In many tubules brush borders were intact and the appearance was normal except for slight dilatation and flattening of the epithelium. Groups of tubules adjacent to obliter-

ated glomeruli had undergone marked atrophy and dilatation (Fig. 13). Others showed hyaline droplet change, hydropic swelling, and rarely necrosis (Fig. 14).

The stroma was edematous throughout and patchy fibrosis was evident in accordance with glomerular and tubular atrophy. There were no infiltrations with inflammatory cells. The pelvic mucosa was intact. The larger vessels, as a rule, appeared normal. Occasionally, the arcuate arteries showed cushion-like fibro-elastic thickening of the intima. The majority of the interlobular arteries appeared intact but here and there edema and beginning necrosis of the intima with deposition of fibrinoid material underneath the endothelium were noted (Figs. 15 and 16). Frequently, fat and lipophages were seen between the internal elastica and the endothelium (Figs. 17 and 18). In the afferent glomerular arteries these changes were common and often were associated with occlusion of the lumen by fibrinoid thrombi and with necrosis of the muscularis and destruction of the internal elastic layer.

Microscopic examination of the lungs revealed marked edema, extensive formation of asphyxial membranes, focal hemorrhage, and multiple foci of pneumonia.

The sternal bone marrow was somewhat less cellular than usual for the patient's age. Hemopoietic elements constituted approximately 50 per cent of the tissue. The remainder was occupied by fat cells and small islands of acellular, edematous fibrils. Marrow from the lumbar vertebrae consisted mostly of hemopoietic elements occurring in small islands. Among these cells, leukopoietic elements predominated.

Case 2

C. R., a 3-year-old Negro boy, underwent a laparotomy in August, 1946, because of the presence of a tumor mass in the left flank. Prior to operation repeated urinalyses had given normal results; the blood urea nitrogen was 9.5 mg. per cent. The blood counts were within normal range. A large embryoma arising from the left kidney was removed. Histologic examination of the kidney showed normal structure.

Between October, 1946, and February, 1947, three courses of radiation therapy were given over the region of the left kidney, alternating front and back on alternate days with a portal of 15 by 15 cm. The conditions of exposure were the same as in case 1, with a daily dosage of 200 r. measured in air, but the dosage for the first course was 2400 r., while in the second and third courses 1600 r. and 1200 r., respectively, were given.

The patient was readmitted because of fatigue, nausea, abdominal pain, pallor, and dyspnea in April, 1947. At that time the urine had a low specific gravity and contained large amounts of albumin, and occasional red cells, leukocytes, and casts. There was a severe normochromic anemia. The blood urea nitrogen was 80 mg. per cent. The clinical picture was that of renal failure, hypertension, and cardiac failure, and the patient died within a few hours.

Post-mortem Examination. Autopsy failed to disclose neoplastic tissue

or metastatic lesions anywhere. The right kidney was of average size, weighing 57 gm. and measuring 7.3 by 4.5 by 3.0 cm. It was only moderately firm, and the capsule was not adherent. The small vessels appeared engorged and made a fine pattern on the pale tan surface. On cut surface, the cortical pattern was obliterated and the parenchyma everted slightly. A few reddish dots of pinpoint size were noted, which seemed to be engorged glomeruli. The medullary portions, the pelvis, ureter, and bladder were not remarkable.

The heart was enlarged, with right ventricular dilatation and left ventricular hypertrophy, and weighed 118 gm. as compared to an average weight of 73 gm. for this age. The liver was enlarged and appeared fatty. The spleen weighed 64 gm., almost twice the normal, and was somewhat soft. Moderate amounts of clear fluid were present in the serous cavities.

The microscopic picture of the kidney was similar to that observed in case 1 (Figs. 19 and 20). Again the outstanding features consisted in glomerular lesions involving chiefly endothelium and basement membranes; formation of thick fibrous "caps" compressing the tufts; obliteration and hyalinization of the loops; tubular atrophy, degeneration and necrosis; and interstitial edema and fibrosis. Again the larger arteries showed localized fibro-elastic thickening of the intima (Fig. 21). In contrast to case 1, the arterioles had not undergone necrosis. Nevertheless, many glomeruli showed infarction of the loops, infiltration of felt-like material with red blood cells, and necrosis of the endothelium. In case 2, a larger proportion of glomeruli had undergone complete obliteration and, accordingly, there was a greater degree of tubular atrophy and fibrosis. No fatty material could be demonstrated in the glomeruli, and there were no macrophages. In some glomeruli a fibrin-like exudate was seen in the capsular spaces and with more advanced lesions of this type fibroblastic organization of the exudate had led to obliteration of the capsular space. Sometimes fibrin was deposited also between the basement membrane and the parietal epithelium of Bowman's capsule. This change often led to proliferation of a segment of epithelium as well as invasion by fibroblasts, so that thick crescents were formed. However, these crescents did not fuse with the tufts, so that capsular spaces were preserved until the obliteration of the tufts was almost complete.

The testes showed extreme tubular atrophy, hyalin-like degeneration of the epithelium, and marked fibrosis. Many seminiferous tubules could be identified only by the concentric condensation of collagen fibrils which had once surrounded them. The remainder had lost their spermatogonia and consisted of poorly preserved Sertoli cells (Fig. 23).

The appearance of the bone marrow varied from section to section

and corresponded to the gross findings. In sections of left ribs 6 to 11, right 4th and 5th ribs, and the lumbar vertebrae which grossly appeared pale, yellow-gray, the marrow was found to consist chiefly of fat and contained only small scattered islands of hemopoietic cells embedded in a gelatinous matrix (Fig. 22). Elsewhere 30 to 50 per cent of the marrow consisted of active hemopoietic tissue. Erythropoiesis seemed significantly decreased.

Case 3

A. Z., an 18-months-old white boy, was admitted because of the accidental discovery of a large mass in the left flank in December, 1948. Intravenous pyelograms indicated good function of both kidneys. Urinalysis gave normal results. Laparotomy was performed and a retroperitoneal neuroblastoma was removed without disturbing the kidney or adrenal. Between December, 1948, and April, 1949, the patient received two series of x-ray treatments. The daily dosage was 200 r. measured in air delivered through four portals in the region of the kidneys, posterior, anterior, left and right lateral, over a field measuring 6 by 10 cm. through a 0.5 mm. Cu and 1.0 mm. Al filter, 200 kv., 10 ma. at 50 cm. skin distance. The first course was given within a period of 3 weeks with a total dosage of 3250 r. A second course following the same technic was interrupted occasionally, so that 18 treatments were given over a period of 36 days with a combined dosage of 3600 r. The total dosage received was 6850 r. Three weeks after the last treatment, the patient was readmitted because of listlessness, tendency to bruise easily, and edema of face and extremities. At that time there was evidence of severe anemia, hypertension, azotemia, albuminuria, and slight hematuria. The patient died within 5 days after readmission.

Post-mortem Examination. There was no evidence of recurrence or metastasis of the tumor. The kidneys were enlarged equally and had a combined weight of 129.5 gm. They were firm, somewhat adherent, and mottled red. On cut surface the cortical markings were obliterated, but in some areas the glomeruli were unusually prominent. The renal vessels, pelves, ureters, and bladder appeared normal.

The heart was hypertrophied. The lungs were edematous and there were large amounts of free fluid in the serous cavities of the body.

Microscopically, the two kidneys were alike and their appearance was similar to that in case 1. The only appreciable difference was the absence of changes in the larger arteries. Although scattered glomeruli were normal in size and structure, the majority presented evidence of severe injury. Many of the capillary tufts were greatly enlarged and the same lack of cellularity described in cases 1 and 2 was noted. The injury involved chiefly the endothelium and the basement membranes, the disintegration of which caused obstruction of capillary loops. Except for isolated glomeruli in which red blood corpuscles were trapped, apparently because of hemorrhage, the loops were bloodless (Figs. 24 and 25). The changes in the parietal layer of Bowman's capsule were less pronounced than those of case 1 but lesions of the same types were

present. Both the isolated hyaline thickening and the cap-like masses of fibrous or hyaline material eccentrically placed opposite the hilum were seen. Evidence of injury to the epithelium of the glomerular capsules was minimal and adhesions were not a part of the lesion. A small number of glomeruli were replaced completely by spherical scars.

Tubular atrophy with proportionate fibrous replacement was present. Tubular dilatation had occurred in a few areas and hydropic and hyaline degeneration accompanied by necrobiosis were seen in a few foci. The small arterioles adjacent to the glomeruli were uniformly and severely damaged and many of them showed necrosis of their walls with obstruction of the lumina. Although there was some intimal thickening in the large vessels, this change was not significant in degree.

Microscopically, the lungs revealed evidence of severe passive congestion and marked edema.

The adrenal glands were within the limits of normal histologic variation.

The spleen presented a normal microscopic pattern except that it was deficient in lymphoid tissue. The germinal centers of the lymph follicles were small, relatively acellular, and often contained hyaline masses. The zones of lymphocytes about them were incomplete and very narrow.

The testes revealed moderate tubular atrophy and an increase in fibrous tissue. The changes did not approach in severity those of case 2.

Comment

Histologic analysis of the renal lesions indicated a pathologic process involving the majority of the glomeruli and tubules but sparing scattered small areas throughout. The changes were too widespread to permit conclusions to be drawn from the distribution of the intact areas. The lesions could not be attributed to structural changes in the small arteries because in case 2 no arteriolar damage was demonstrable and in cases 1 and 3 the glomerular changes usually seemed to have preceded the appearance of the arteriolar lesions. The glomerular changes appeared to be primary or, at least, to be independent of either vascular or tubular lesions. The tubular damage was in the main proportional in degree, age, and extent to the destruction of the glomeruli. The presence of foci of tubular necrosis, however, suggested the possibility of an additional direct insult to the tubular epithelium.

Several different stages of glomerular as well as of tubular lesions could be recognized. Side by side one found complete scarring, glomerular obstruction of long standing, recent degenerative changes, and acute necrosis. These findings suggested recurrent injury to the kidneys.

Study of the glomerular lesions indicated that the damage involved primarily the endothelium and the basement membrane. These were the structures which showed degeneration or necrosis in the early phases of the process. By contrast the epithelium remained in good condition and adhesions across the capsular spaces were lacking.

The changes in the intralobular arteries and the afferent vessels in cases 1 and 3, consisting in edema, fatty degeneration, and necrosis of the intima, seemed to be analogous to the glomerular lesions, and probably the result of the same agent which caused them. The vascular lesions could not be held responsible for the development of the glomerular changes, nor secondary to the presence of renal failure and hypertension. In summary, the histologic features were those of a glomerulonephritis but differed from the ordinary forms of that condition. In place of endothelial proliferation there was necrosis and degeneration of the endothelium. Inflammatory cellular infiltrations and epithelial adhesions were lacking, and capsular fibrosis was eccentric, of unusual severity, and apparently independent of changes within the tuft. In regard to the correlation of the histologic findings with the clinical picture, the presence of glomerular obstruction and extensive tubular damage readily explained the development of renal failure and hypertension.

We believe that the changes described by us constitute a distinctive picture. Even if this were not conceded, the parallel development of nephritis in three children receiving radiation therapy requires consideration of the possible etiologic rôle of roentgen rays. A number of arguments make such a relationship appear likely.

1. In the first 2 cases, the normal structure of the kidney removed prior to radiation excluded the existence of an earlier generalized renal lesion. In case 3, normal kidney function prior to radiation was proved by pyelograms and urinalysis and by the normal blood pressure.

2. The survival periods, reckoned either from the onset or from the time of completion of radiation therapy, were remarkably similar in the 3 cases (Table I).

TABLE I

Data on Dosage and on Elapsed Time for Three Children Developing Glomerulonephritis Following X-Radiation of the Renal Area

Case	Age	Nephrectomy	Total dosage	Size of portal	Duration of life from start of radiation to death	Duration of life from completion of radiation to death
	yrs.		*r.*	*cm.*	*mos.*	*wks.*
1	4	Unilateral	5850	15 x 15	7	9
2	3	Unilateral	5200	15 x 15	6½	7–8
3	1½	None	6850	6 x 10	5	4½

3. In cases 1 and 2, although radiation was applied primarily to the region of the extirpated kidney, the portal area was large and the remaining kidney must have received a substantial amount of radiation. In case 3 both kidneys were in the area of exposure. Hypoplasia of the marrow in distant parts of the skeleton in cases 1 and 2 (data on case 3 were not available) and atrophy of the testis in cases 2 and 3 testify to the dissemination of radiation effects in the body.

4. The similarity of the histologic picture in the 3 cases suggests an entity, and the differences between this picture and that of the usual variants of glomerulonephritis in childhood indicate an unusual pathologic process and make likely a special cause.

5. The lesions resemble the changes produced with radiation in dogs by Hartman, Bolliger, and Doub.[2]

The comparison of the human lesions with the experimentally produced lesions in animals requires great caution. The reports of experimental studies on the effect of roentgen rays on the kidney are contradictory. This is not surprising since different workers have used different technics, dosages and time intervals, different species of animals, and variable experimental conditions. Although the earliest investigators failed to demonstrate renal damage after radiation, probably because the animals died or were killed before sufficient time had elapsed for the appearance of lesions, later authors have been able to produce significant changes in the kidneys of a variety of animals. However, some reports described chiefly tubular, others glomerular, and still others vascular lesions. Bolliger and Laidley,[3] in attempting to clarify the confusion resulting from the variety of histologic pictures attributed to radiation effects, distinguished between two main groups of experiments. According to them, authors working with heavily filtered high voltage radiation produced chiefly glomerular and vascular changes while those employing lightly filtered or unfiltered rays of low or medium voltage observed predominantly tubular destruction.

The conditions existing in our patients approximate those of the first group, of which the experiments of Hartman, Bolliger, and Doub [2] are outstanding examples. These authors described glomerular obliteration, thickening of Bowman's capsule, increase in interstitial connective tissue, tubular atrophy and dilatation, and endarteritis. Judging from the illustrations, the similarity between this picture and that seen in our cases was marked. This impression was confirmed by one of the authors of the experimental study.[4]

None of the experiments performed by various workers were quite comparable to the conditions existing in our cases. Apart from the

differences in species, exposure, and dosage, the animal experiments were different in that they were performed on fully grown subjects whereas our patients were young children in an active phase of growth. It is conceivable, and in fact probable, that the kidneys of the growing organism may be more sensitive to radiation than those of the mature individual.

A further feature which was not duplicated in most of the published experiments was the removal of one kidney prior to irradiation in 2 of our patients. The remaining kidney was thus exposed to the effects of the roentgen rays at a time when it was undergoing hypertrophy because of marked compensatory increase in function. This may have rendered it more susceptible to radiation injury.

We do not attempt to explain the absence of vascular lesions in the small arteries in one of the cases. Radiation effects are said to involve vessels in which endothelium forms a proportionately large part of the wall, and to damage endothelium somewhat selectively.[5] The striking involvement of the glomerular capillary endothelium was in keeping with this theory. The fact remains, however, that the typical vascular changes seen after radiation [6] in arterioles and small arteries were not present in our cases. The problem posed by these observations should be capable of solution by experimental methods duplicating the clinical situations described.

The literature dealing with clinical or pathologic observations on renal damage from radiation in humans is meager. Domagk [7] was probably the first to observe renal lesions after exposure to roentgen rays. His patient was a 9-year-old girl who received 2 exposures of 2 skin erythema doses each, the second erroneously without filtration. There was erythema and vesication of the skin, and an almost immediate reaction characterized by albuminuria and cylindruria. The patient died 6 months later with symptoms of renal failure. The glomeruli were largely obliterated by connective tissue. Many contained fat, Bowman's capsule was greatly thickened, often compressing the tufts. The tubules were dilated, atrophic or necrotic, or had undergone fatty changes. The intima of the larger vessels showed fatty degeneration and thickening by connective tissue. The description given by Domagk and the survival time of his patient were similar to those of our cases. No other comparable cases seem to have been recorded.

Dean and Abels [8] examined a surgically removed kidney 7 years after radiation of its lower pole. The patient, a 20-year-old girl, had received radiation over the left kidney area because of an abdominal mass thought to be due to Hodgkin's disease. Seven years later hypertension devel-

oped. The left kidney was removed. Its lower pole was shrunken and microscopically showed "extreme atrophy and sclerosis." Hypertension subsided after the operation.

Mertz, Howell, and Hendricks [9] removed the kidney of a 29-months-old male infant who had received a total of 16,335 r. in divided doses in nine courses because of a tumor. The kidney showed destruction of the vascular and secretory elements of the portions uninvolved by tumor. The patient survived and the remaining kidney had apparently adequate renal function.

Hagner and Coleman [10] radiated the renal area of a 45-year-old male using a filter, a distance of 70 cm., and a current of 1200 ma. The total dosage was given over a period of 4 days. Twenty days later nephrectomy was performed. The patient seemed well until 10 days later when anuria developed and he died shortly. An autopsy was not performed.

Doub, Bolliger, and Hartman [11] observed albuminuria, cylindruria, a rise in non-protein nitrogen of the blood, and an elevation of the blood pressure in 2 adults who had received radiation over the renal area. They also reported the answers to a questionnaire prepared by them and sent to pathologists and radiologists: 14 of 143 replies from pathologists and 2 of 65 from radiologists indicated that "nephritis" had developed after radiation, presumably over the renal area.

Munger [12] stated that he had observed depression of renal function after radiation with subsequent restoration after cessation of therapy.

Waters [13] administered radiation therapy in daily doses up to 345 r. and total doses up to 3500 r. over three or four portals to a number of adults with hypernephromas and found normal glomeruli and only slight swelling of the tubules when the kidneys were removed within the following 5 months.

The review of the literature is far from conclusive in regard to harmful effects of therapeutic radiation in the kidneys. Since radiation is a commonly used procedure in the treatment of renal embryomas of children, one would expect others to have reported findings similar to ours if the lesions were related to radiation injury. This is all the more probable because the poor prognostic outlook of the primary disease justifies maximal dosages. We shall not attempt to interpret the dearth of such reports at this time. Our own report is made in the hope of stimulating further clinical and morphologic studies of human kidneys exposed to radiation and of animal experiments duplicating therapeutic conditions.

In our patients no recurrence of tumor was found and it may be assumed that they would have survived had it not been for the development of nephritis and renal insufficiency. If these phenomena were relat-

ed to radiation injury, as we are inclined to believe, the relationship should be explored and the potential dangers should be fully understood. The hypothesis is advanced that radiation injury may have been the cause of the lesions and that the renal tissues of young, growing individuals may be more susceptible to radiation effects than those of the full grown organism. The literature to date contains no conclusive proof of serious radiation injury to human kidneys after conventional therapeutic doses. The animal experiments published thus far fail to duplicate closely the therapeutic conditions existing in children, but lesions similar to those described in our patients have been produced with similar technics.

Summary

Three cases of nephritis of an unusual type developing in children who had received therapeutic radiation over the renal area are reported with clinical and pathologic descriptions. Prior to radiation therapy the kidneys had been normal in structure and function as proved by examination of surgical specimens in 2 of the cases and by functional tests in the third case. The patients died with the clinical picture of renal failure and hypertension. The survival periods in relation to both beginning and completion of radiation were similar in the 3 cases.

The renal lesions were unique in appearance and were interpreted as evidence of injury to endothelium and basement membranes of the glomerular capillaries and Bowman's capsules. There also were tubular atrophy, degeneration and necrosis, and interstitial fibrosis. The basic picture was identical in the 3 cases and developed within a similar time interval after radiation.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 144

FIG. 1. Case 1. Low-power view of right kidney, showing glomerular enlargement, obstruction, hemorrhage, scarring, and tubular atrophy with interstitial fibrosis. Hematoxylin and eosin stain. × 84.

FIG. 2. Case 1. High-power view of typical glomerular lesion, showing enlargement and partial fusion of capillary loops, and obstruction of lumina with fibrillar material. The epithelial lining is intact. × 550.

FIG. 3. Case 1. Smaller glomerulus showing hydropic swelling of capillary loops; also beginning necrosis. There is necrosis of the afferent arteriole. × 550.

FIG. 4. Case 1. Glomerulus showing marked irregular thickening and spongy disintegration of the basement membrane. Azocarmine stain. × 550.

FIG. 5. Case 1. Glomerular loops partially filled with lipophages. × 550.

FIG. 6. Case 1. Glomerulus showing fibrinoid necrosis of basement membrane as well as obstruction of capillary lumina by fibrillar material. Of note again is the preservation of the epithelium. × 550.

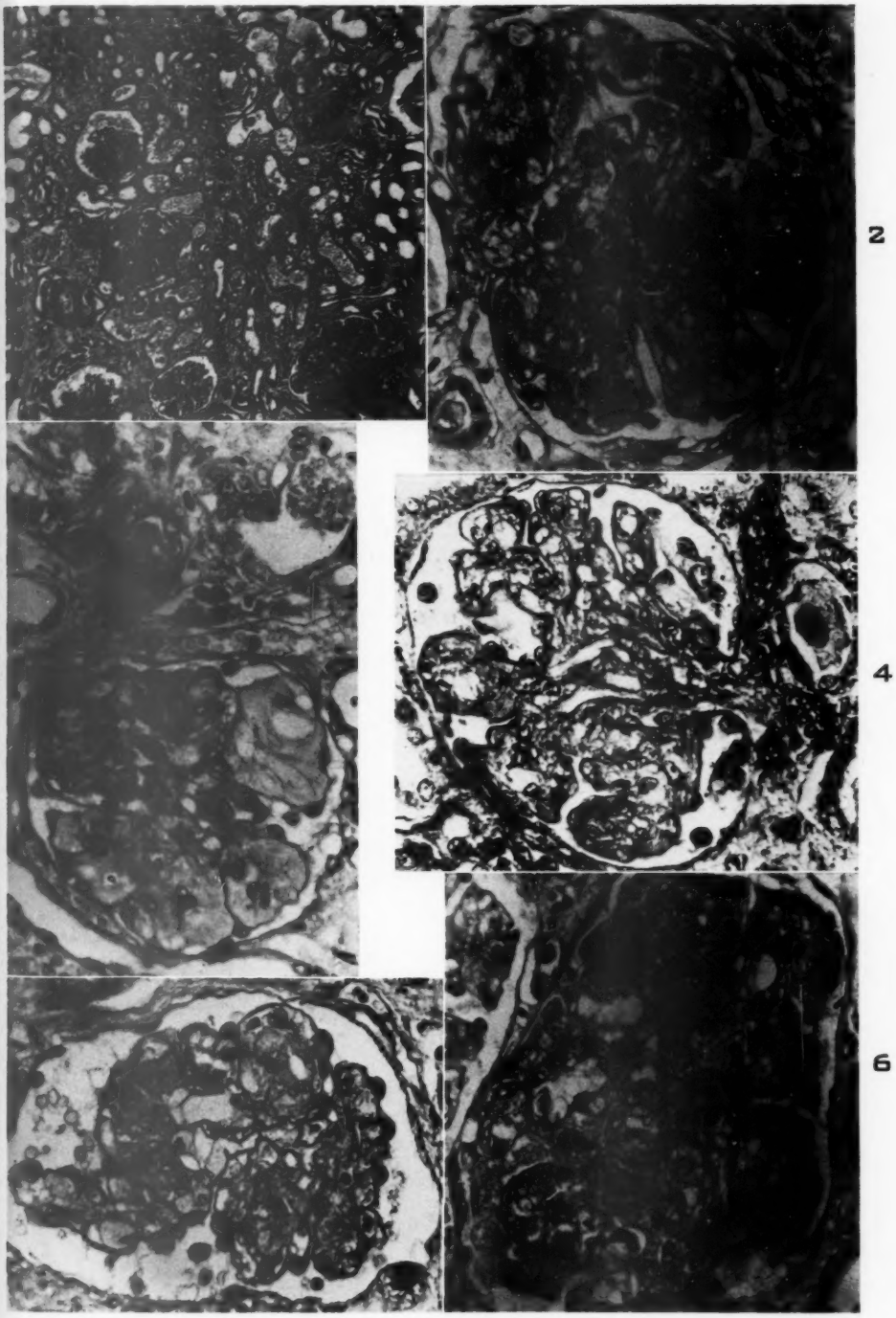

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PLATE 145

FIG. 7. Case 1. Greatly enlarged hemorrhagic and necrotic glomerulus. There is a lack of hemorrhage into the capsular space and partial preservation of the epithelium. × 550.

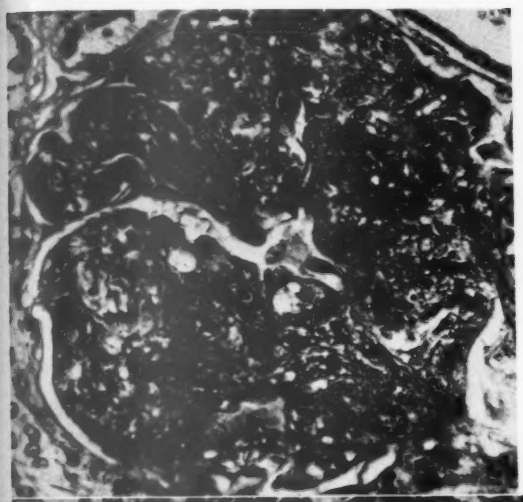
FIG. 8. Case 1. Two smaller glomeruli, apparently of normal size, showing marked hyaline thickening of the basement membrane of Bowman's capsule in the hilar region compressing the base of the tufts. Masson's tetrachrome stain. × 550.

FIG. 9. Case 1. Localized thickening of the basement membrane of Bowman's capsule opposite hilus. Van Gieson's stain. × 550.

FIG. 10. Case 1. More advanced thickening of Bowman's capsule from a cap-like mass compressing atrophic glomeruli. × 550.

FIG. 11. Case 1. Another glomerulus with cap-like thickening of Bowman's capsule. Of note is the preservation of the original outline of the tuft and the lack of epithelial adhesions. × 550.

FIG. 12. Case 1. Final stage of glomerular scarring. × 550.



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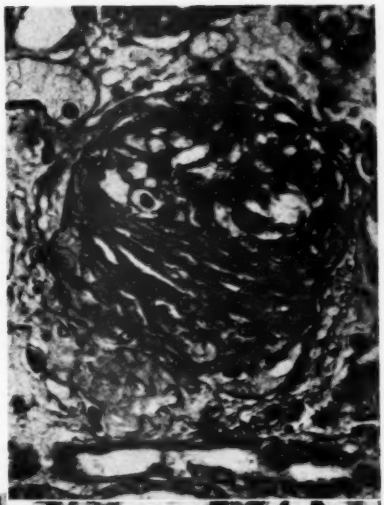

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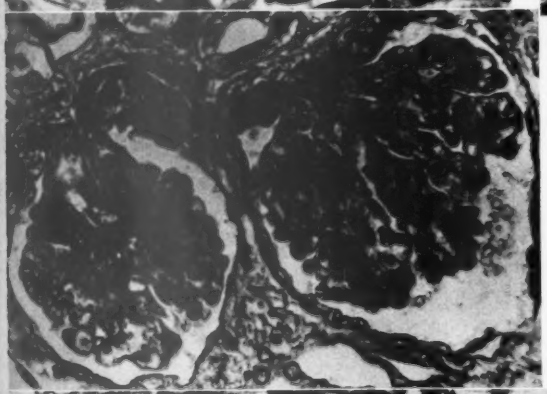

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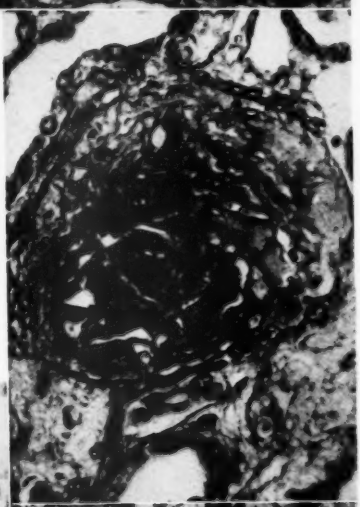

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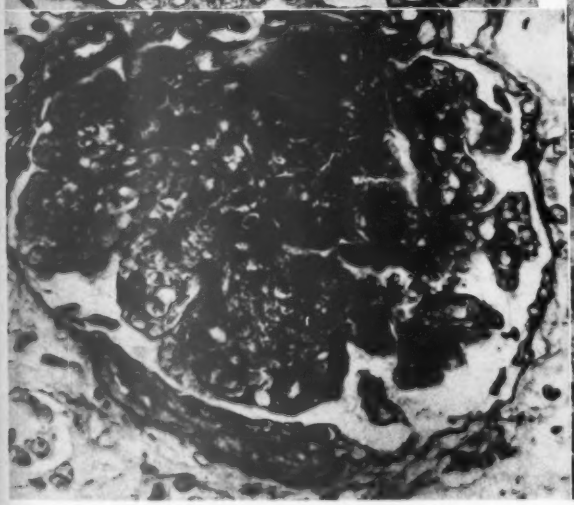

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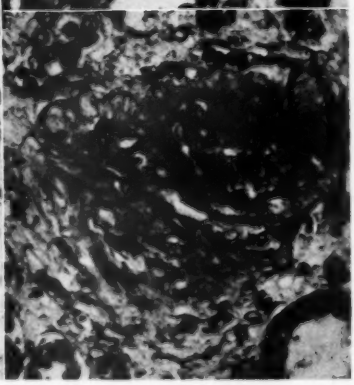

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PLATE 146

FIG. 13. Case 1. Tubular dilatation and atrophy. Lumina are filled with granular precipitate. × 550.

FIG. 14. Case 1. Localized area of tubular necrosis. × 550.

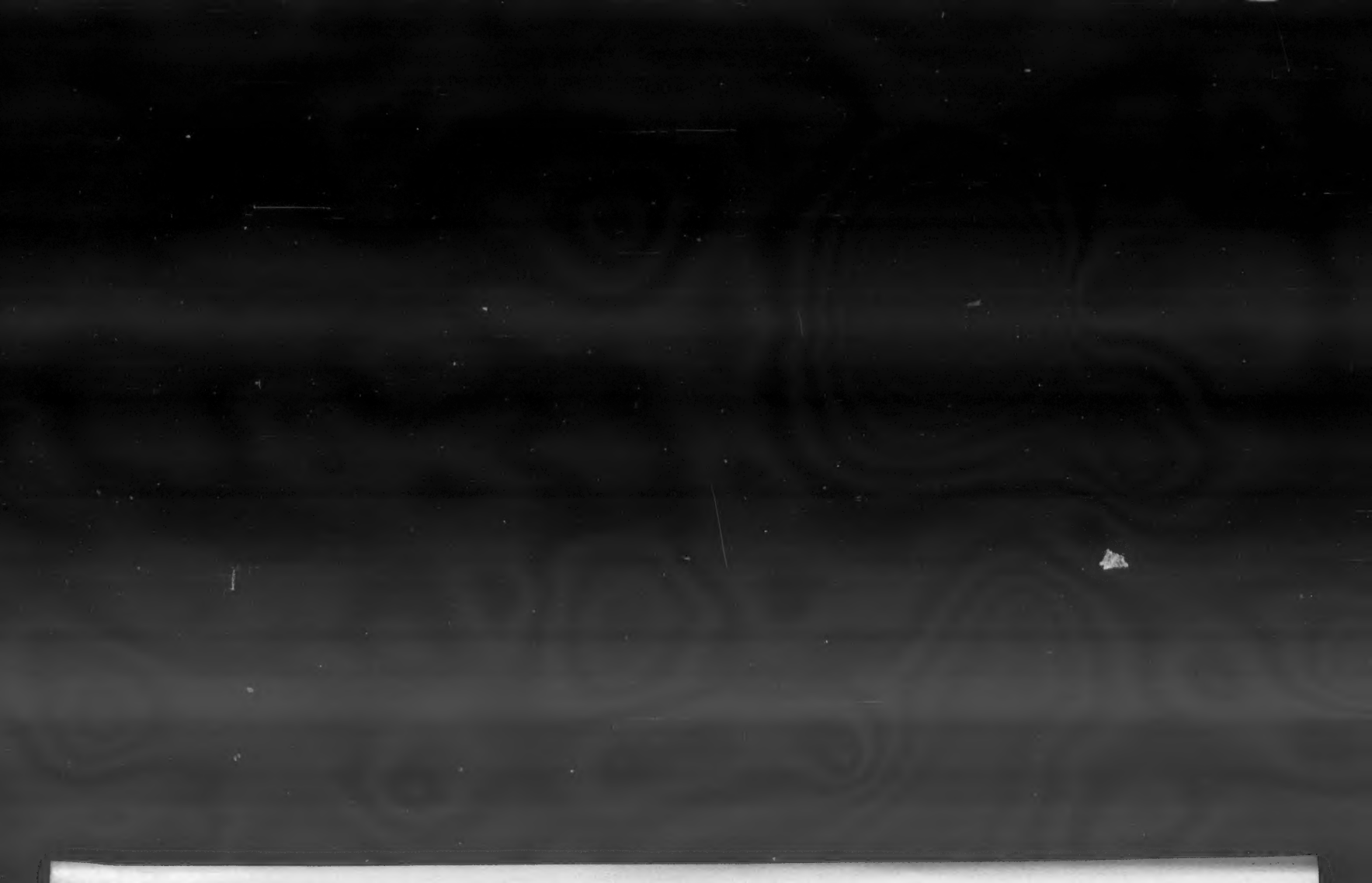
FIG. 15. Case 1. Complete necrosis of afferent arteriole. × 550.

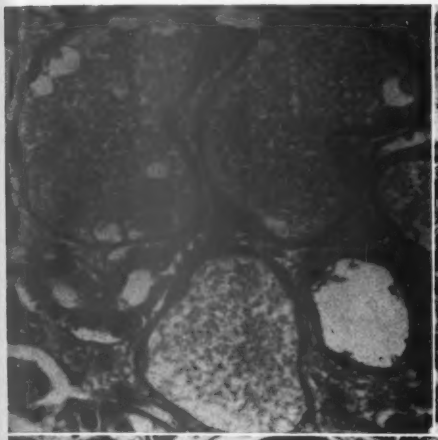
FIG. 16. Case 1. Typical vascular lesion, showing tremendous edema of intima, almost completely separating the lumen, and necrosis of endothelium. × 550.

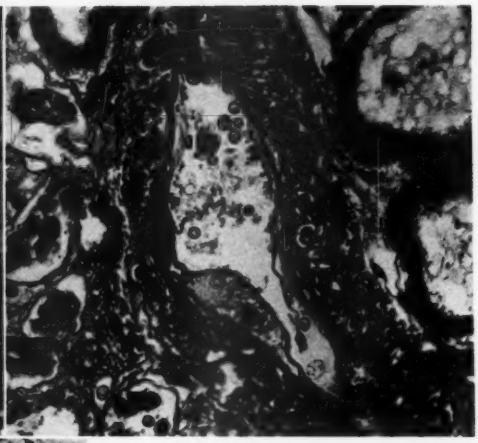
FIG. 17. Case 1. Localized area of intimal degeneration in a small artery. There is deposition of fatty material and a lipophage in the intima. × 550.

FIG. 18. Case 1. Vessel showing intimal necrosis and edema at one end and lipophages beneath the endothelium at the other end. × 320.

FIG. 19. Case 2. Low-power view of remaining kidney removed at autopsy, showing fibrosis, glomerular scarring, hypertrophy, hemorrhage, and tubular atrophy. × 84.







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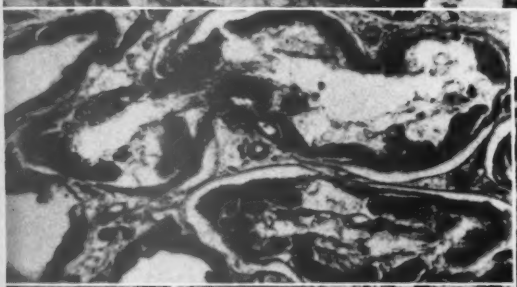

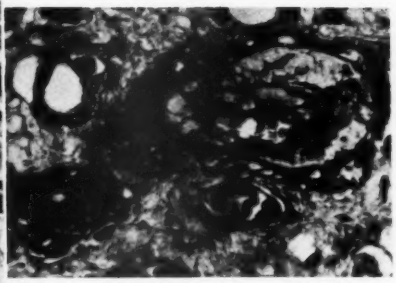

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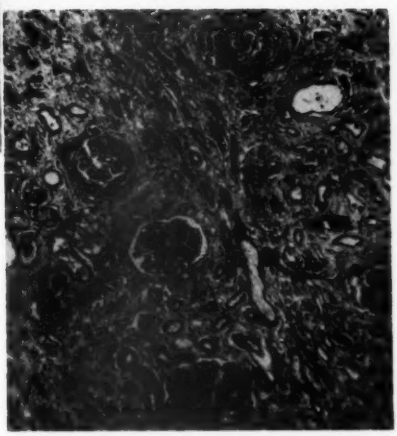

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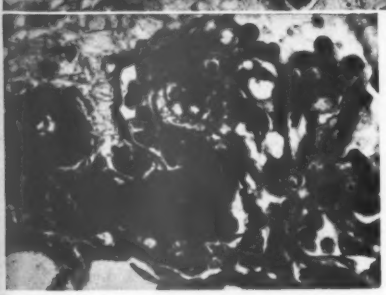

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PLATE 147

FIG. 20. Case 2. Hemorrhagic glomerulus with deposition of fibrin in the capsular space. × 550.

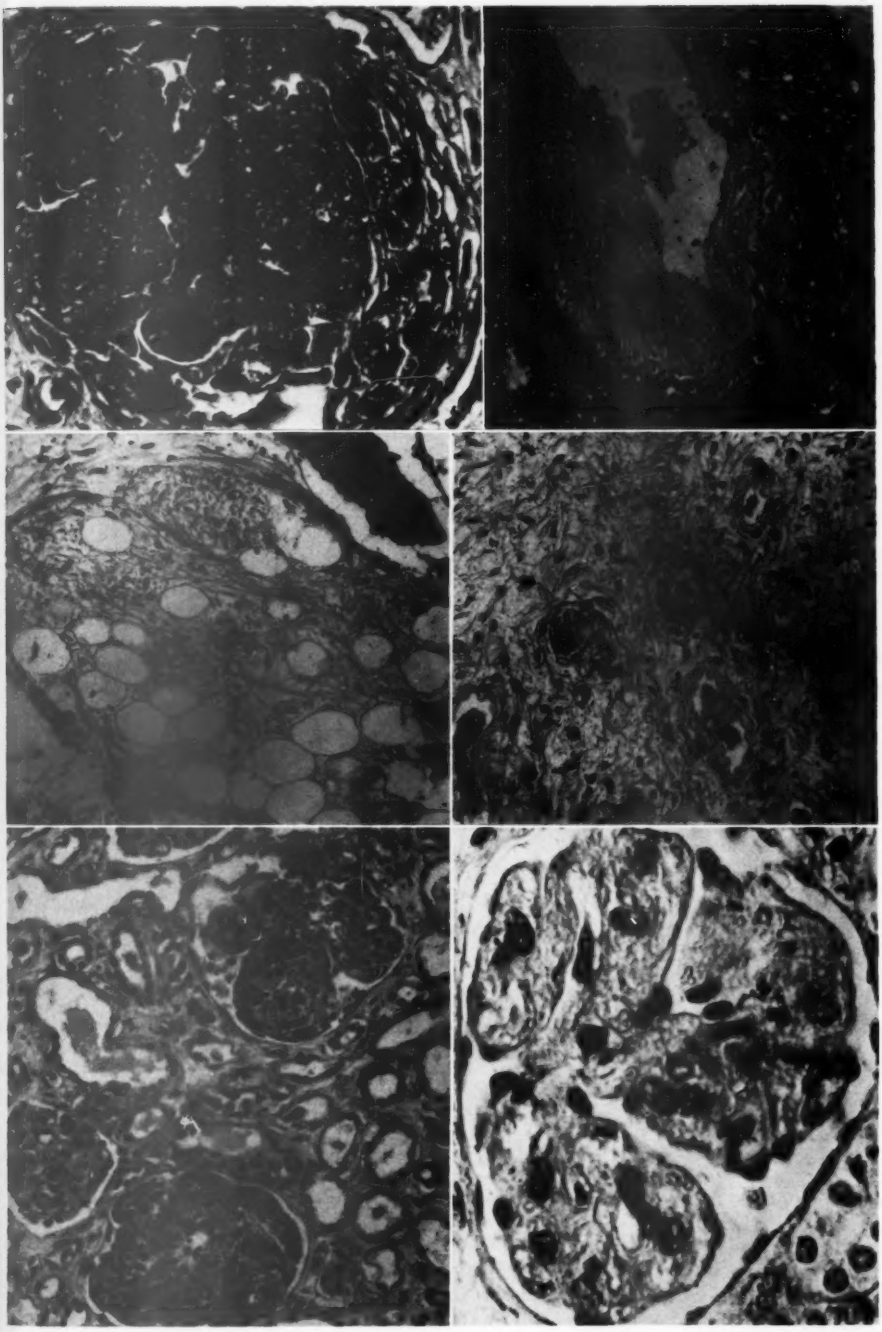
FIG. 21. Case 2. Intimal fibrosis, and splitting and duplication of internal elastic layer in a large renal artery. × 160.

FIG. 22. Case 2. Bone marrow from lumbar vertebrae, showing edematous, fibrillar connective tissue and fat. The dark cells scattered here and there are for the most part erythrocytes. × 160.

FIG. 23. Case 2. Testis with extreme atrophy of tubules, and fibrosis. The lining of the few remaining tubules is composed of poorly stained Sertoli cells. × 160.

FIG. 24. Case 3. Right kidney, showing large hypocellular glomeruli without fusion with Bowman's capsule, tubular atrophy, and increase in interstitial fibrous tissue. The changes in the left kidney were the same.

FIG. 25. Case 3. High-power view of a glomerulus. Here are seen bloodless loops, fusion of loops, marked thickening and disintegration of basement membranes and endothelium, with normal epithelium in the parietal layer of Bowman's capsule.



Zuelzer, Palmer, and Newton Glomerulonephritis in Young Children

THE PATHOLOGY OF FATAL CARBON TETRACHLORIDE POISONING WITH SPECIAL REFERENCE TO THE HISTOGENESIS OF THE HEPATIC AND RENAL LESIONS *

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The pathologic changes in 12 cases of fatal poisoning due to carbon tetrachloride are reported. For some time [1-9] centrilobular necrosis of the liver and nephrosis have been recognized as characteristic findings in carbon tetrachloride toxicity. However, the wide variations in the histologic picture generally have not been recognized. Peery reported 3 fatal cases of carbon tetrachloride poisoning showing variations in the degree of hepatic damage.[4] In the present group, the interval between exposure and death varied from 4 to 18 days, thus affording an excellent opportunity to study the morphologic changes at various stages of development. There was a definite correlation between the length of survival and the pathologic changes at autopsy, particularly in the liver and kidney.

Seven persons ingested carbon tetrachloride accidentally or with suicidal intent; 5 inhaled the fumes of carbon tetrachloride. No significant differences were observed in the lesions resulting from these two types of exposure. All but one of the 12 persons included in this study had acute or chronic alcoholism.

SYMPTOMS AND SIGNS

The first manifestations of poisoning were nausea and vomiting beginning usually within 24 hours. These symptoms occurred immediately in 4 individuals who ingested the carbon tetrachloride and in one person who was exposed to the fumes over a period of several hours. Abdominal pain was associated with severe nausea and vomiting. Two of those who inhaled the fumes had severe headaches.

Jaundice developed within 2 to 4 days in 11 individuals; there was no jaundice in one who survived for only 4 days after ingestion of carbon tetrachloride. The icterus index varied from 16 to 125 units. In general, the greater the degree of jaundice, the shorter was the period of survival.

* Sponsored by the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the author are a result of his own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

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TABLE I

Carbon Tetrachloride Poisoning

Case	Mode of poisoning	Alcohol	Onset of symptoms	Initial symptoms	Jaundice	Renal failure	Duration of illness
1. B.J. Male Age 38	Ingestion	Chronic alcoholic	Immediately	Nausea, vomiting, and abdominal pain	Absent		4 days
2. R.B. Male Age 73	Ingestion	Intoxicated			Severe; onset unknown		4 days
3. C.E. Male Age 54	Ingestion	Recent alcoholic bouts	Immediately	Nausea and vomiting	Severe; within 2 days	Anuria within 3 days	7 days
4. F.S.G. Male Age 38	Inhalation	Chronic alcoholic	Within 24 hours	Nausea, vomiting, and malaise	Severe; onset unknown	Oliguria in 4 days; anuria in 5 days	8 days
5. E.C. Female Age 30	Ingestion	Recent alcoholic bouts	? (Psychotic)		Moderate; onset unknown	Oliguria in 3 days; anuria in 5 days	8 days
6. R.T. Male Age 46	Ingestion	Chronic alcoholic	Within few hours	Abdominal pains and cramps	Moderate; within 4 days	Oliguria within 5 days	10 days
7. J.B.R. Male Age 41	Inhalation	None	Immediately	Nausea, vomiting, and abdominal pain	Absent	Oliguria in 2 days; anuria in 8 days	11 days
8. H.C. Male Age 52	Ingestion	Chronic alcoholic; intoxicated	Within few hours	Nausea, vomiting, and abdominal pain	Moderate; within 3 days	Anuria in 2 days	11 days
9. R.J.H. Male Age 45	Ingestion	Intoxicated	Immediately	Nausea	Moderate; within 3 days	Oliguria in 24 hrs.; anuria in 30 hrs.	12 days
10. A.W. Male Age 29	Inhalation	Intoxicated	Within 12 hours	Nausea and vomiting	Slight; transient; onset unknown	Anuria in 3 days	15 days
11. E.M.B. Male Age 32	Inhalation	Intoxicated	Within 24 hours	Headache, generalized pains, nausea and vomiting	Slight; transient; onset within 3 days	Oliguria in 2 days	16 days

In the 3 who survived for more than 2 weeks the jaundice was slight and transient.

The renal function was not known in the 2 persons who survived for only 4 days. Oliguria and anuria occurred within 2 to to 4 days in 10 persons. Renal function did not return to normal in any; however, in 2 individuals urine of low specific gravity was passed following several days of complete anuria. The levels of blood urea nitrogen and creatinine were markedly elevated at the time of death, repeatedly reaching ten times the normal values. The levels of chloride and potassium in the blood ranged from below normal to above normal.

Pulmonary edema occurred as a late manifestation and was followed usually very shortly by death. Various procedures such as application of tourniquets, administration of hypertonic glucose solutions, and phlebotomy afforded temporary relief.

Pathologic Findings

The liver, kidneys, and lungs were consistently the sites of pronounced abnormalities. The morphologic variations in these organs showed a definite correlation with the length of the survival period. The gross and microscopic findings are presented in condensed form for each of the 12 cases.

Case 1

Liver. Gross: Enlarged; lobular markings exaggerated; speckled yellow and red; soft. Microscopic: Acute central necrosis and hemorrhage; severe fatty degeneration.

Kidneys. Gross: Swollen and pale; one kidney weighed 260 gm. Microscopic: Proximal convoluted tubules with swollen, pale, faintly granular "hydropic" epithelium; distal convoluted tubules, loops of Henle, and collecting tubules normal except for occasional granular casts.

Lungs. Moderate edema of lower lobes.

Case 2

Liver. Gross: Normal size; lobules accentuated; speckled yellow and reddish brown, soft. Microscopic: Acute central necrosis and hemorrhage; "ghost cells," focal polymorphonuclear infiltration; moderate fatty degeneration of remaining hepatic cells.

Kidneys. Gross: Slightly swollen and icteric; one kidney weighed 125 gm. Microscopic: Glomeruli collapsed, capsular epithelium swollen; proximal convoluted tubules with coarsely granular swollen epithelium; distal convoluted tubules with flat epithelium; bile-stained and heme casts in loops of Henle, distal convoluted and collecting tubules.

Lungs. Moderate edema of lower lobes.

Case 3

Liver. Gross: Small; flabby; wrinkled capsule; yellowish green; lobules accentuated. Microscopic: Lobules collapsed; condensation of central reticular framework; remaining hepatic cells large with occasionally vacuolated pale cytoplasm; lymphocytes, macrophages, hemosiderin and bile pigment in degenerated areas.

Kidneys. Gross: Swollen; icteric; two times normal size. Microscopic: Glomeruli collapsed; distended subcapsular space; swelling of capsular epithelium; "hydropic" and coarsely granular epithelium of proximal convoluted tubules; basophilic concretions and pigment casts of distal convoluted tubules, loops of Henle, and collecting tubules; minimal cortical and medullary lymphocytic infiltration; interstitial edema.

Lungs. Severe congestion and mild edema.

Pancreas. Atypical pancreatitis.

Case 4

Liver. Gross: Normal size; wrinkled capsule; flabby; yellowish green; accentuated lobular markings. Microscopic: Lobules collapsed; condensation of central reticular framework; remaining hepatic cells large, occasional cytoplasmic vacuoles; lymphocytes, macrophages, and hemosiderin in degenerated centers of lobules.

Kidneys. Gross: Swollen and pale; 1½ times normal size. Microscopic: Glomeruli collapsed; dilated capsular space filled with precipitate; swollen epithelium of Bowman's capsule; cells of proximal convoluted tubules cuboidal with pale, sparsely granular cytoplasm; cells of distal convoluted tubules low-cuboidal with granular cytoplasm; pigment and cellular casts of loops of Henle, distal convoluted tubules, and collecting tubules; focal lymphocytic infiltration of cortex and medulla; congestion of medulla; interstitial edema.

Lungs. Severe edema of all lobes; acute necrotizing bronchitis; bronchiolitis and early bronchopneumonia.

Peritoneum. Ascites.

Case 5

Liver. Gross: Small; wrinkled capsule; flabby; yellowish green and brown; lobular markings accentuated. Microscopic: Lobules collapsed; condensation of reticulum in degenerated centers of lobules. Remaining hepatic cells large, occasionally binucleate; rare mitotic figures; cytoplasm pale and occasionally vacuolated; lymphocytes, macrophages, hemosiderin, and bile pigment in centers of lobules.

Kidneys. Swollen, soft, pale yellow; two times normal size. Microscopic: Glomeruli collapsed; dilated periglomerular space filled with granular precipitate; capsular epithelium swollen; proximal convoluted tubule with granular cuboidal, regenerating epithelium; distal convoluted tubules with low regenerating epithelium; numerous basophilic concretions and heme casts in loops of Henle, distal convoluted tubules, and collecting tubules; focal lymphocytic infiltration of cortex and medulla; congestion of medulla; interstitial edema.

Lungs. Marked edema and congestion of all lobes.

Heart. Myocytes and focal myocarditis, slight.

Thorax. Bilateral hydrothorax.

Case 6

Liver. Gross: Normal size, smooth capsule; flabby; yellowish green and brown; lobular markings accentuated. Microscopic: Lobules collapsed; condensation of reticular framework in degenerated centers of lobules; remaining hepatic cells large, frequently binucleate; occasional mitotic figures; abundant faintly granular cytoplasm with occasional large vacuoles; few lymphocytes and macrophages in central degenerated areas.

Kidneys. Gross: Swollen, soft, pale; one kidney weighed 340 gm. Microscopic: Glomeruli collapsed; granular precipitate in dilated periglomerular space; capsular epithelium occasionally swollen; epithelium of proximal convoluted tubules swollen, with "hydropic" cytoplasm; epithelium of distal convoluted tubules regenerating; numerous basophilic concretions and pigment casts in loops of Henle, distal con-

voluted tubules, and collecting tubules; focal perivascular lymphocytes and plasma cell infiltration of medulla; interstitial edema.

Lungs. Massive edema of all lobes.

Heart. Myocytes and focal myocarditis, slight.

Case 7

Liver. Gross: Normal size and consistency; yellowish tan with reddish brown centers of lobules. Microscopic: Lobules collapsed with central reticular condensation; remaining hepatic cells slightly larger than is normal, with granular cytoplasm; occasional binucleate cells; a few fat vacuoles; rare lymphocytes and occasional macrophages in centers of lobules.

Kidneys. Gross: Swollen, pale, and soft; one kidney weighed 230 gm. Microscopic: Glomeruli normal; capsular epithelium and periglomerular space normal; proximal convoluted tubules with slightly pale granular epithelium and slight dilatation; pigment casts, basophilic concretions and regeneration of distal convoluted tubules, loops of Henle, and collecting tubules; focal lymphocytic infiltration and congestion of medulla; interstitial edema.

Lungs. Severe edema of all lobes.

Thorax. Bilateral hydrothorax.

Case 8

Liver. Gross: Normal size, smooth capsule; flabby; yellowish green and brown; lobular markings accentuated. Microscopic: Collapsed lobules with central condensation of reticulum; remaining hepatic cells large, frequently binucleate; abundant faintly granular cytoplasm with few large vacuoles; focal collections of lymphocytes, macrophages, hemosiderin, and bile pigment.

Kidneys. Gross: Swollen, soft and pale yellow; one kidney weighed 310 gm. Microscopic: Glomeruli with moderate amount of blood; capsular epithelium swollen; epithelium of proximal convoluted tubules swollen with pale, sparsely granular cytoplasm; degeneration and regeneration of epithelium, pigment and cellular casts and occasional basophilic concretions of loops of Henle, distal convoluted tubules, and collecting tubules; focal interstitial lymphocytes and plasma cells of cortex and medulla; congestion of medulla; interstitial edema.

Lungs. Moderate edema and congestion of all lobes.

Pancreas. Atypical pancreatitis.

Case 9

Liver. Gross: Normal size; slightly wrinkled capsule; flabby; yellowish green and brown; lobular markings accentuated. Microscopic: Lobules collapsed with central condensation of reticular framework; remaining cells slightly enlarged with finely granular cytoplasm; occasional large cytoplasmic vacuoles; frequent binucleate cells; occasional lymphocytes and rare macrophages, scanty hemosiderin and bile pigment in centers of lobules.

Kidneys. Gross: Swollen, pale, icteric; one kidney weighed 395 gm. Microscopic: Glomeruli with scanty blood; periglomerular space distended with granular precipitate. Epithelium of proximal convoluted tubules varying from cuboidal granular cells to columnar pale cells, marked nuclear variation and some regeneration; distal convoluted tubules dilated, low epithelium; some regeneration of epithelium of distal convoluted tubules, loops of Henle, and collecting tubules; numerous pigment casts; focal lymphocytic infiltration, often perivascular; interstitial edema.

Lungs. Marked edema of all lobes.

Pancreas. Atypical pancreatitis.

Peritoneum. Ascites.

Case 10

Liver. Gross: Slightly enlarged; soft; yellowish green and tan; lobular markings accentuated. Microscopic: Small residual areas of central necrosis filled with condensed reticulum; hepatic cells large with abundant granular cytoplasm and occasionally multiple nuclei and mitotic figures; few lymphocytes, macrophages and hemosiderin in centers of lobules.

Kidneys. Gross: Swollen, pale, and soft; one kidney weighed 390 gm. Microscopic: Glomeruli collapsed; cells of proximal convoluted tubules swollen, coarsely granular cytoplasm; numerous pigment and cellular casts, rare basophilic concretions, and regenerating epithelium and occasional dilatation of loops of Henle, distal convoluted tubules and collecting tubules; focal lymphocytic infiltration of cortex and medulla; occasionally vasculitis.

Lungs. Massive edema of all lobes; early bronchopneumonia.

Pancreas. Atypical pancreatitis.

Case 11

Liver. Gross: Normal size and consistency; pale tan with dark brown flecks accentuating centers of lobules. Microscopic: Very small residual areas of central necrosis occupied by condensed reticular framework; hepatic cells larger than is normal, with faintly granular cytoplasm; rare lymphocytes and macrophages containing hemosiderin.

Kidneys. Gross: Swollen, soft and pale; one kidney weighed 250 gm. Microscopic: Glomeruli with little blood; cells of proximal convoluted tubules large with pale, sparsely granular cytoplasm; pigment casts, desquamated cells, basophilic concretions, regeneration of epithelium, and occasional dilatation in loops of Henle, distal convoluted tubules, and collecting tubules; focal mild lymphocytic infiltration of cortex and medulla.

Lungs. Massive edema of all lobes; early bronchopneumonia.

Pancreas. Atypical pancreatitis.

Case 12

Liver. Gross: Normal size and consistency; tan with dark green flecks; lobular differentiation accentuated. Microscopic: Very small residual areas of central necrosis; some condensation of reticulum in centers of lobules; hepatic cells slightly larger than is normal, with faintly granular cytoplasm; rare lymphocytes, and macrophages in centers of lobules.

Kidneys. Gross: Swollen and slightly pale; one kidney weighed 240 gm. Microscopic: Glomeruli with scant blood, often collapsed; slight dilatation of periglomerular space, capsular cells swollen; cells of proximal convoluted tubules swollen and hydropic; pigment and cellular casts; basophilic concretions, and regeneration of epithelium in loops of Henle and distal convoluted tubules; slight focal lymphocytic infiltration of cortex and medulla.

Lungs. Massive edema of all lobes; early bronchopneumonia.

Pancreas. Atypical pancreatitis.

Liver

In summary, the liver was enlarged in 2 individuals; in one, death occurred 4 days after exposure, and in the other, 15 days after exposure. In the remainder, the liver was normal in size or smaller than is normal. The lobular differentiation was exaggerated in all. Several of the livers presented a "nutmeg" appearance on gross examination and were soft and flabby.

The microscopic appearance showed striking variations. In the 2 individuals who died in 4 days, the hepatic lobules were considerably larger than is normal. There were very extensive necrosis and some hemorrhage involving the centers of the lobules. Many times "ghost cells" without nuclei and with indistinct cytoplasmic outlines were present. Occasional small groups of polymorphonuclear leukocytes were present. The areas of central necrosis were more or less confluent leaving only a narrow rim of recognizable but abnormal hepatic cells adjacent to the portal areas. These cells were two to three times normal size and usually contained large amounts of fat within their cytoplasm. The cytoplasm between the vacuoles of fat was coarsely granular. The nuclei were often distorted; binucleate hepatic cells often were present. The reticular framework of the lobules was intact even in the areas of central necrosis. Because the individual hepatic cells were enlarged, the reticulum envelopes of the individual cells were also larger than is normal.

In the livers of the 7 persons who died between 7 and 12 days, the lobules were smaller than in those described above. There were moderately large central areas of necrosis. The polymorphonuclear leukocytes present in the earlier stage were replaced by macrophages and occasional lymphocytes. Some of the macrophages contained hemosiderin. There were vacuoles of fatty material in the necrotic areas as well as in the cytoplasm of some of the hepatic cells at the periphery of the lobules. These cells were somewhat larger than is normal and their cytoplasm was usually coarsely granular. The nuclei were large although somewhat variable in size. Some of the cells contained multiple nuclei; occasional mitotic figures were present. The reticular framework of the centers of the lobules was compressed. The individual reticulum envelopes were still present but were much smaller in the centers of the lobules than is normal.

In the 3 persons who died from 15 to 18 days after poisoning, the hepatic lobules were approximately normal in size. The areas of central necrosis were very small and in some of the lobules were identified only by the presence of a few macrophages containing hemosiderin and rare lymphocytes. The individual hepatic cells were larger than is normal. These cells had pale to dark granular cytoplasm. There were occasional multinuclear cells; a few mitotic figures were present. There were no cytoplasmic vacuoles. The reticular framework of the lobules was practically normal except for tiny areas in the centers of the lobules where there was condensation of reticulum.

Thus, there was a progressive diminution in the size of the areas of necrosis with longer periods of survival. In no case was there prolifera-

tion of fibroblasts or bile ducts. In some of the more acute poisonings, a considerable amount of bile pigment was present in the centers of the lobules; there was a minimal amount of bile pigment, or none, in those who survived for longer periods.

Kidney

The kidneys were swollen and pale and occasionally were icteric. The weight of a single kidney was nearly 400 gm. in 2 individuals. The cortical and medullary striations were more prominent than is normal.

As in the hepatic lesions, there was marked variation in the character and degree of the renal changes. The glomeruli showed no specific lesions, although often the glomerular capillaries were devoid of blood. The capsular spaces often were distended and filled with finely granular precipitate. The lining cells of Bowman's capsule frequently were swollen.

The earliest striking change in the kidneys occurred in the proximal convoluted tubules. In those who died in 4 days the epithelium of the proximal convoluted tubules was swollen. The cytoplasm was pale and slightly granular, giving these cells the appearance of "hydropic degeneration." Fat stains revealed small amounts of fat as fine droplets within the cytoplasm. The rest of the renal parenchyma was of normal appearance.

In the later stages (7 to 12 days) the epithelium of the proximal convoluted tubules varied from the early "hydropic appearance" to that of severe cloudy swelling with marked granularity of the cytoplasm. Occasionally there was evidence of regeneration characterized by low cuboidal cells, irregular nuclear spacing, and rare mitotic figures. The distal convoluted tubules were markedly altered. These frequently contained basophilic concretions and reddish brown, hyaline, or infrequent cellular casts. The epithelium was nearly always low-cuboidal with variable nuclear spacing and occasional mitotic figures. The loops of Henle showed similar changes. There was some lymphocytic infiltration of the supporting tissue of both cortex and medulla.

In persons who survived for more than 2 weeks (15 to 18 days), the renal changes were similar to those seen in persons surviving for shorter periods. However, heme casts and basophilic concretions were less numerous and the inflammatory reaction in the interstitial tissue was more severe. There was marked edema of the stroma. "Tubulo-venous thromboses" were not found.

Lungs

Edema was pronounced in nearly all lungs, which were voluminous and very heavy. The bronchi usually were filled with frothy fluid. The cut surfaces were extremely edematous and somewhat congested. Bronchopneumonia was seen in 4 persons. In 3 of these, the pneumonia was mild and made evident by only a few polymorphonuclear leukocytes in some of the alveoli. The severity of the edema was roughly proportional to the length of the survival period. In one case (no. 4) there was an acute necrotizing bronchitis, bronchiolitis, and early bronchopneumonia; this individual had been exposed to carbon tetrachloride fumes for 1½ days while spraying engines in the hold of a ship.

Pancreas

In 5 persons there was dilatation of many of the acini and flattening of the acinar epithelium. In some areas there was partial disruption of the acinar wall and associated autolysis of adjacent tissue. There was slight polymorphonuclear leukocytic and some lymphocytic infiltration adjacent to these areas.

Heart

In many individuals there were occasional myocytes in the interstitial tissue of the myocardium and in 2 there were focal accumulations of lymphocytes.

Discussion

The synergistic effect of alcohol upon the toxicity of carbon tetrachloride has been known for some time.[10] This explains the remarkably frequent occurrence of alcoholism in the present group of fatal poisonings. While the effect of alcoholism in producing either acute or chronic fatty changes in the liver is well known, its effect on the kidneys is not so well understood. I have frequently observed a considerable degree of cloudy swelling and nuclear irregularity in the epithelium of the proximal convoluted tubules in alcoholic individuals.

The occurrence of very severe hepatic lesions in those dying very shortly following exposure to carbon tetrachloride indicates that acute hepatic insufficiency was in all likelihood the most important factor in causing death. The possibility of these persons having had abnormal livers prior to exposure to carbon tetrachloride must be considered.* With increasingly longer periods of survival, the hepatic lesions were

* There were 2 additional cases not included in this series in which the individuals died during exposure to carbon tetrachloride fumes. Both showed severe congestion of all viscera and a few perivascular hemorrhages of the brain. One of these persons had a history of chronic alcoholism and showed a fatty liver.

progressively less evident. This is considered to be due to two factors; namely, a less severe initial hepatic injury, and rapid regeneration of hepatic tissue following the single episode of acute injury. The clinical observations of slight and transient jaundice in these latter cases are in keeping with this interpretation.

The sequence of morphologic changes of the liver are considered to be as follows: (1) fatty degeneration and centrilobular necrosis, (2) centrilobular hemorrhage and infiltration by acute inflammatory cells, (3) histiocytic infiltration of necrotic areas, (4) collapse of centers of lobules resulting in condensation of the reticular framework in these areas, and (5) regeneration of hepatic tissue from remaining hepatic cells. However, there appears to be considerable overlapping of these various processes.

Histogenetically, the renal lesions were of great interest. They were not limited to the "lower nephron" and, indeed, the only striking morphologic alterations in the kidneys of those surviving for 4 days occurred in the proximal convoluted tubules and Bowman's capsule. The "hydropic degeneration" occurring in the proximal convoluted tubules apparently is closely associated with an imbalance in electrolyte and water metabolism. Similar changes have been noted in persons with disturbances in electrolyte and water metabolism not associated with carbon tetrachloride poisoning.[11] Follis, Orent-Keiles, and McCollum[12] and Lowenhaupt and Greenberg[13] have described lesions in the renal tubules associated with potassium and chloride deficiencies. McManus and Rutledge[14] recently reported a decrease in the alkaline phosphatase content of cells of the proximal convoluted tubules in cases of "crush syndrome." In later stages the "hydropic degeneration" of the proximal tubules was partially obscured by the more striking picture of heme and cellular casts, basophilic concretions, severe cloudy swelling, necrosis and regeneration of the distal convoluted tubules, and the interstitial inflammatory reaction. In those persons surviving for longer than 2 weeks, the heme casts and basophilic concretions were diminished in number, but many of the distal convoluted tubules, loops of Henle, and collecting tubules were lined by regenerating epithelium. Dilatation of some of the tubules was noted. Thus, the sequence of morphologic changes appears to be as follows: (1) degeneration of proximal convoluted tubules, (2) degeneration of the distal convoluted tubules, loops of Henle, and collecting tubules, and the appearance of heme and cellular casts and basophilic concretions, (3) appearance of inflammatory cells in the supporting connective tissue, and (4) regeneration of distal convoluted tubules, loops of Henle, and collecting tubules.

Within certain limits there was an inverse relationship between the severity of the hepatic lesions and the severity of the renal lesions.

The dilatation of the pancreatic acini and focal areas of pancreatitis are essentially similar to the changes reported by Baggenstoss [15] in the pancreases of some patients dying in uremia.

Summary and Conclusions

The frequent occurrence of a history of alcoholism in cases of fatal carbon tetrachloride poisoning indicates a synergistic nephrotoxic as well as hepatotoxic effect between alcohol and carbon tetrachloride.

No essential differences were noted between the lesions occurring as a result of inhalation and those due to ingestion of carbon tetrachloride.

The hepatic lesions in persons dying soon after exposure were very extensive. The severity of the hepatic lesion diminished with longer periods of survival. This is believed to be due to two factors, namely, less severe injury initially and rapid regeneration following injury.

The renal lesions in persons dying within a few days after exposure were relatively slight and involved primarily the proximal convoluted tubules. The renal morphologic changes became progressively more pronounced with longer periods of survival.

An inverse relationship was noted between the severity of the hepatic lesions and the severity of the renal lesions.

Pulmonary edema was present consistently and was greater in those who survived for longer periods.

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DESCRIPTION OF PLATES

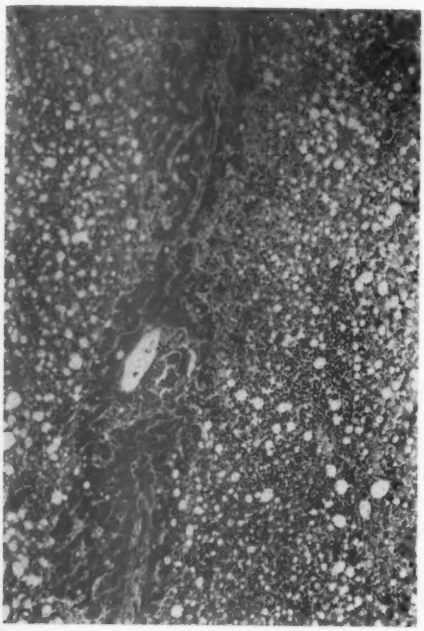
PLATE 148

FIG. 1. Case 2. Massive central necrosis of liver, 4 days after exposure to carbon tetrachloride. There is a narrow zone of altered but viable hepatic cells adjacent to portal and perilobular areas. The central necrotic area shows ghost cells, fatty vacuoles, and focal collections of polymorphonuclear cells and hemorrhage. Hematoxylin and eosin stain. × 66.

FIG. 2. Case 5. Massive central necrosis of liver, 8 days after exposure to carbon tetrachloride. There are small groups of viable but abnormal hepatic cells in the portal and perilobular zones. The "ghost cells" have largely disappeared and there has been coalescence of fat droplets to form large pools of fat represented by the vacuoles in the center of the lobule. A few macrophages are present at the periphery of the necrotic zone. Hematoxylin and eosin stain. × 66.

FIG. 3. Case 9. Central necrosis of liver, 12 days after exposure to carbon tetrachloride. The zone of viable and regenerating hepatic cells is considerably larger and the areas of central necrosis smaller than in cases 2 and 5. The central necrotic zone shows some macrophages containing hemosiderin. Hematoxylin and eosin stain. × 66.

FIG. 4. Case 12. Small residual areas of central necrosis of liver, 18 days after exposure to carbon tetrachloride. A few macrophages containing hemosiderin are present in the centers of lobules.



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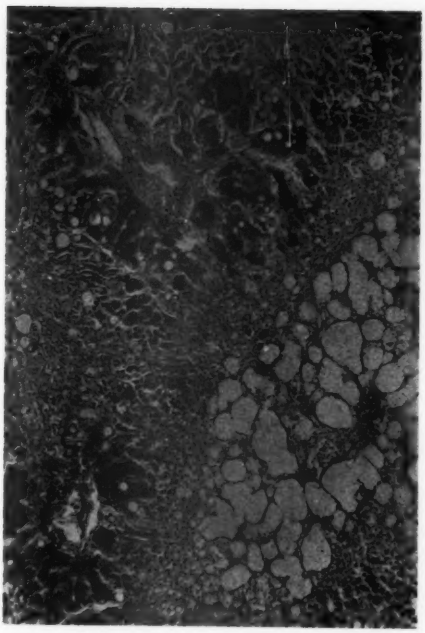

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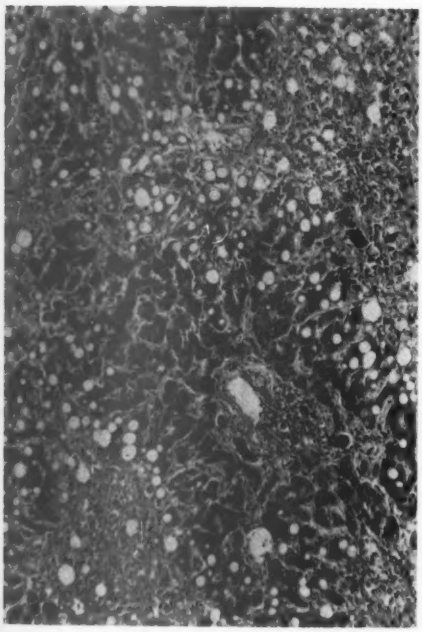

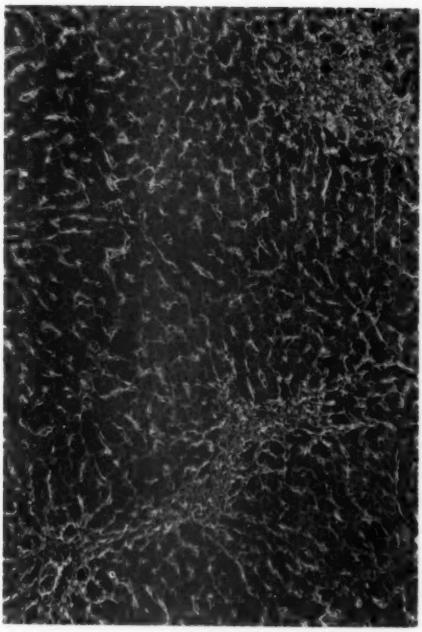

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Moon

Carbon Tetrachloride Poisoning

PLATE 149

FIG. 5. Case 1. Renal cortex, 4 days after exposure to carbon tetrachloride. The epithelium of the proximal convoluted tubules is markedly swollen; the cells have a pale, faintly granular, "hydropic" cytoplasm. The glomeruli and distal convoluted tubules are normal. Hematoxylin and eosin stain. × 110.

FIG. 6. Case 1. Renal medulla, 4 days after exposure to carbon tetrachloride. Occasional granular casts are present. Hematoxylin and eosin stain. × 110.

FIG. 7. Case 5. Renal cortex, 8 days after exposure to carbon tetrachloride. Epithelium of Bowman's capsule shows swelling and increased numbers of nuclei. Collapsed glomeruli; granular precipitate in subcapsular space, proximal and distal convoluted tubules. Severe cloudy swelling and basophilic concretions of tubules. There is some regenerative activity. Hematoxylin and eosin stain. × 110.

FIG. 8. Case 5. Renal medulla, 8 days after exposure to carbon tetrachloride. Numerous heme casts and basophilic concretions; edema and slight lymphocytic infiltration of interstitial tissue. Hematoxylin and eosin stain. × 110.

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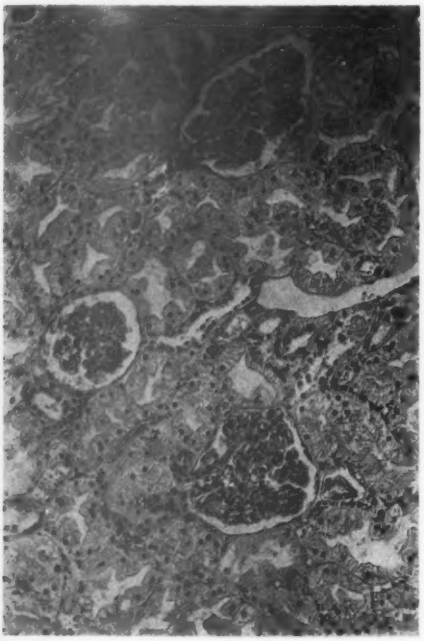

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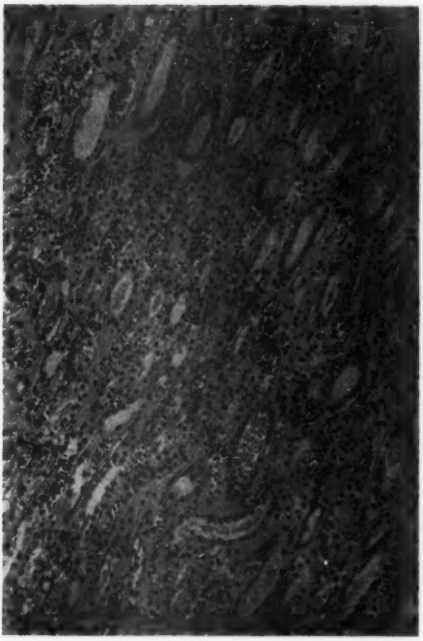

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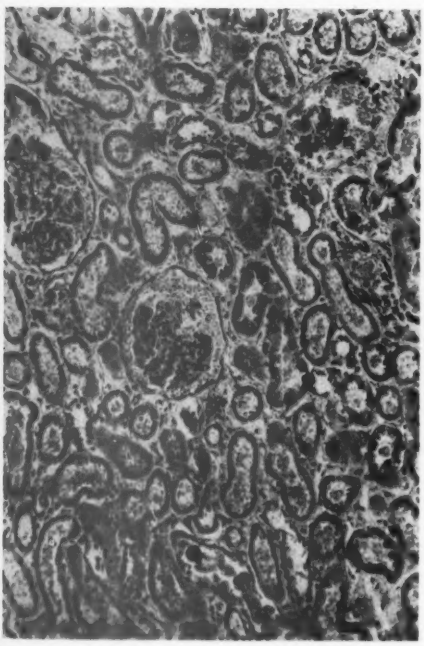

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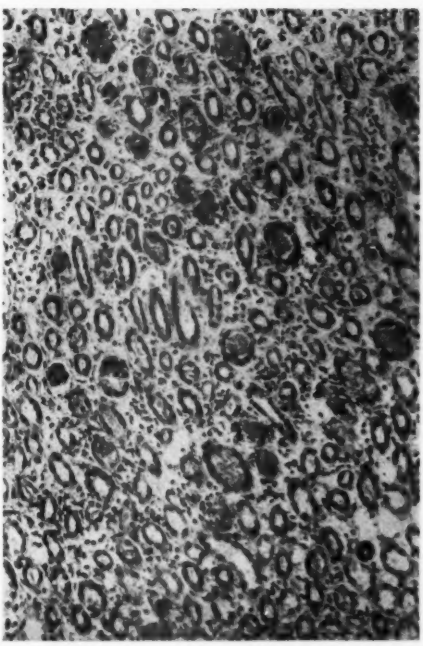


Moon

Carbon Tetrachloride Poisoning

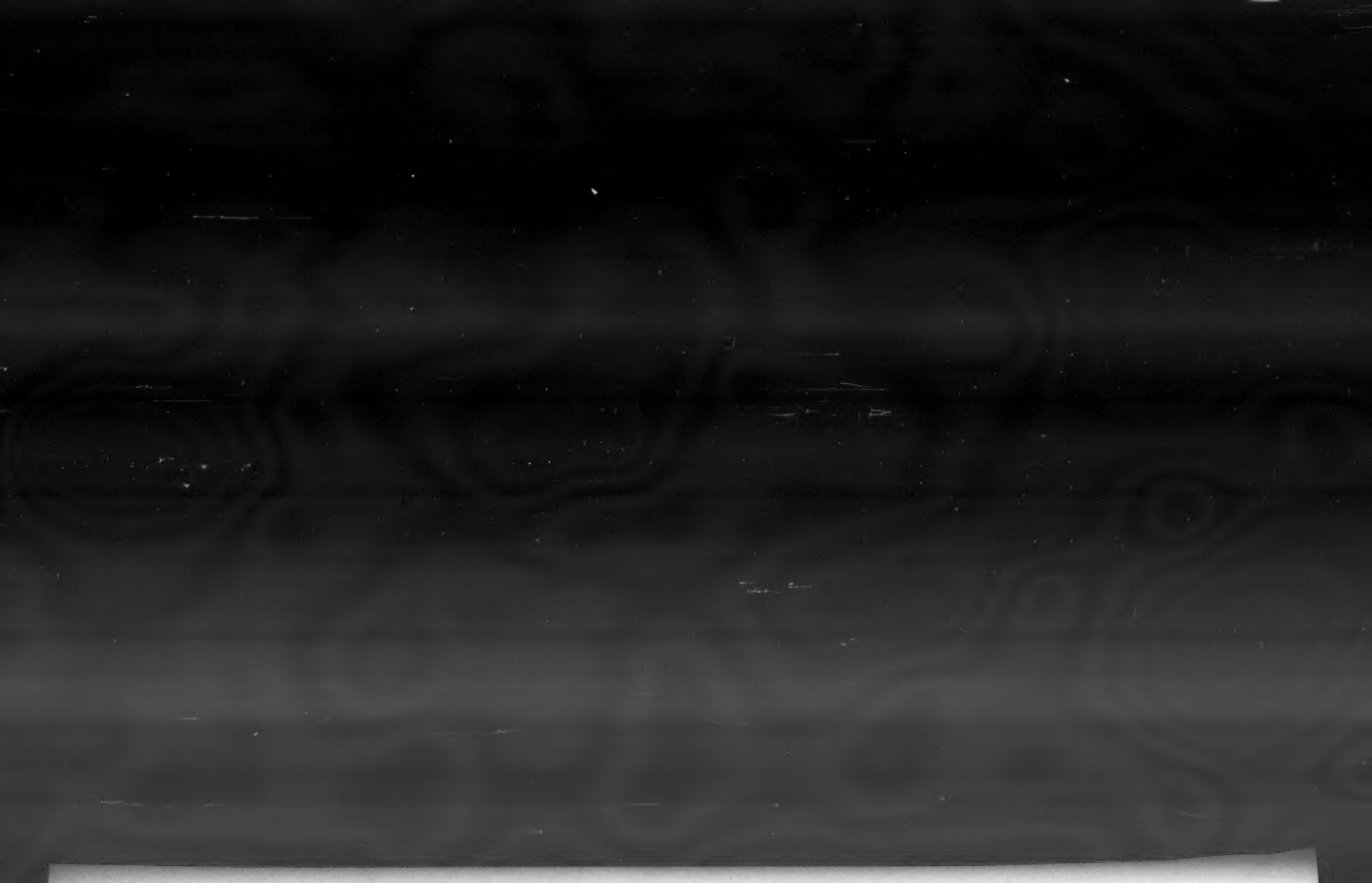
PLATE 150

FIG. 9. Case 12. Renal cortex, 18 days after exposure to carbon tetrachloride. Epithelium of Bowman's capsule is swollen. Epithelium of proximal convoluted tubules has a "hydropic" or coarsely granular, dense cytoplasm. Dilated distal convoluted tubules contain hyaline casts and show regenerating epithelium. Rare to occasional basophilic concretions are present. Hematoxylin and eosin stain. × 110.

FIG. 10. Case 12. Renal medulla, 18 days after exposure to carbon tetrachloride. Regenerating epithelium in loops of Henle and collecting tubules. Edema and moderately severe lymphocytic infiltration of interstitial tissue. Dilated collecting tubule. Hematoxylin and eosin stain. × 110.

FIG. 11. Case 10. Pulmonary edema, 15 days after exposure to carbon tetrachloride. Hematoxylin and eosin stain. × 110.

FIG. 12. Case 10. Pancreas, 15 days after exposure to carbon tetrachloride. Low cuboidal to flat epithelium of acini. Lumina filled with "colloid" and desquamated cells. There is a focal area of autolysis and infiltration by lymphocytes and polymorphonuclear leukocytes. Hematoxylin and eosin stain. × 110.



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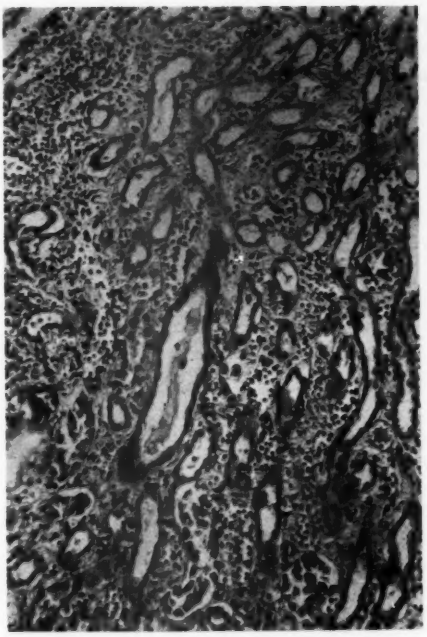

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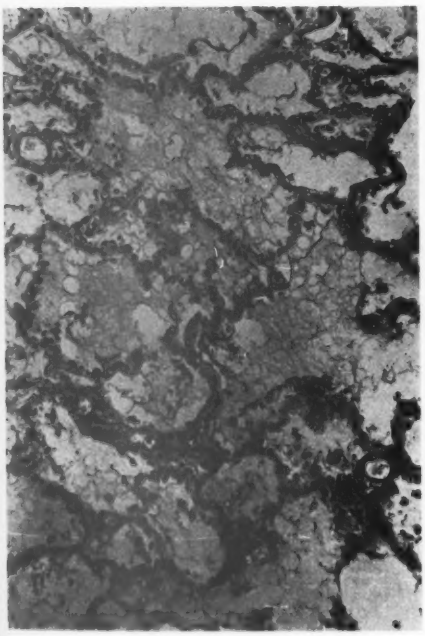

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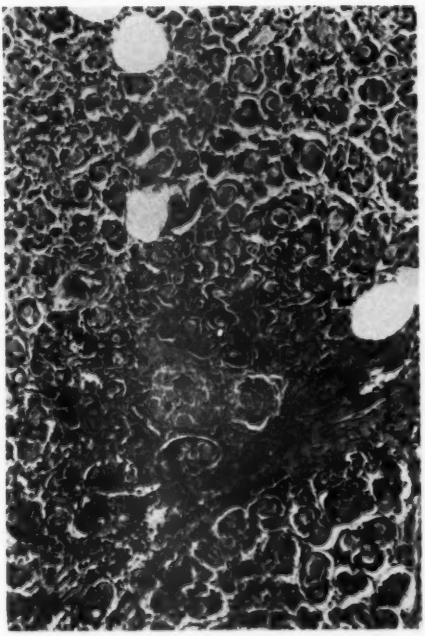

Moon

Carbon Tetrachloride Poisoning

LOCAL GIGANTISM (A MANIFESTATION OF NEUROFIBROMATOSIS): ITS RELATION TO GENERAL GIGANTISM AND TO ACROMEGALY ILLUSTRATING THE INFLUENCE OF INTRINSIC FACTORS IN DISEASE WHEN DEVELOPMENT OF THE BODY IS ABNORMAL *

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Evidence is submitted in this paper in support of the opinion that local gigantism occurs as a manifestation of neurofibromatosis. In this contribution two conditions that are thought to be related to localized neurofibromatosis will be considered briefly, namely, local bone softening and multiple osteochondromata; the main theme, however, is local gigantism and its relation to general gigantism and to acromegaly.

DEFINITIONS

In earlier papers (Inglis [1,2]) the terms "neural intrinsic factor" and "basic intrinsic factor" have been defined and employed and the following definitions are in accord with those used in the earlier contributions. The *neural intrinsic factor* is of intrinsic nature and of neural origin, related to specific nerve sheath tissue (neurilemma or sheath of Schwann); it has several potentialities, one of which is in the direction of neurofibromatosis. The *basic intrinsic factor* is more primitive and more comprehensive than neural intrinsic factor, and is in play from the time of fertilization of the ovum to the time of appearance of neural tissue in the developing embryo; it embraces a potential neural intrinsic factor as a component. The terms *intrinsic factor* and *intrinsic factors*, used without qualification, comprise both neural intrinsic factor and basic intrinsic factor. In regard to the development of particular lesions of the neurofibromatosis complex, it is often difficult to decide whether intrinsic factor is in play at the neural intrinsic factor level or at the basic intrinsic factor level.

LOCAL GIGANTISM

Striking examples of local gigantism may occur in the alimentary tract, but the examples most accessible for study involve the extremities, particularly the digits. Bell and Inglis,[3] in a paper entitled "Plexiform neuroma," referred to a young woman who, as a child, had an abnormal left middle finger (macrodactyly). The length and girth of this finger and the size of the nail were all in proportion; the sole gross abnormality of the digit seemed to be its large size. This finger was amputated in

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early childhood, and there was no record of a microscopic examination. When the patient was 20 years of age her left index finger was much larger than the right. The left ring finger was enlarged also, mainly on its radial side. The left thumb was smaller than the right. At that time the left index finger was amputated and fibrolipomatous tissue removed from the palm. The left median nerve at the wrist was composed of large bundles (Fig. V of that paper); microscopically, these large bundles were found to consist to a considerable extent of fibrous tissue; in transverse section some of them were seen to be composed of an outer zone of dense fibrous tissue and a central part which was cellular (Fig. VI of that paper). Doubt was expressed at the time as to whether the cells in this central part were Schwann cells or connective tissue cells of the endoneurium. This material has been re-examined recently and the doubt still remains, but, whereas the inclination then was to favor mesoblastic connective tissue origin, the inclination now is to think that at least some of these cells may have been neurilemmal cells of epiblastic origin.

In the field of distribution of the neurofibromatous median nerve in that case there was neurofibromatous overgrowth of soft tissues, thickening of walls of blood vessels, and osteochondromata on the phalanges. These changes are thought to be local effects due to the influence of neural intrinsic factor of neurofibromatosis and not due to interference with innervation in the ordinary sense. This interpretation is supported by the fact that Bland-Sutton [4] has recorded with illustrations a similar case of plexiform neuroma affecting the musculospiral nerve and its branches but with no alteration in size of the digits of the affected limb. Two other examples of macrodactyly, and a third case which is thought to be akin to them, will now be described.

Case 1

The patient (P.U.S. 3045) was a woman, 24 years of age. At birth the left second toe was enlarged, especially the distal part. The enlarged distal part was amputated when the patient was 5 years of age, but the stump gradually enlarged and skiagrams showed changes in the bones. The appearance of the foot when the patient was 24 years of age is shown in Figure 1. All toes appeared normal except the second which was enlarged throughout, but especially so at the distal end. The tumor of soft parts of the second toe involved the whole distal end of the toe including the plantar aspect where it extended to the adjacent part of the ball of the foot.

A skiagram of the foot taken at about this time is shown in Figure 2. The two phalanges of the left second toe were enlarged, especially the

second. Presumably the terminal phalanx was removed at the first operation (although this is not specifically stated in the notes, which are very brief). In Figure 2 a small piece of bone is seen on the medial side of the head of the second phalanx. In this skiagram the small piece of bone appeared to be in contact with a bony process projecting from the head of this phalanx, but in a skiagram showing a somewhat lateral view, the small piece of bone appeared to be separated by a short distance from the head of the second phalanx. It is unlikely that this small, apparently separate piece of bone represented a vestigial terminal phalanx, firstly, because of its position at the side of the head of the second phalanx, and secondly, because the enlargement of the two phalanges of this toe increased as the distal end of the digit was approached. It is suggested that this small, apparently separate piece of bone corresponds to the almost separate piece of bone that is illustrated in Figure 6 of the article on the pathologic anatomy of acromegaly by Marie and Marinesco.[5]

When the patient was 24 years of age a second operation was performed, the left second toe and part of the corresponding metatarsal bone being removed, but not the tumor of soft tissues on the ball of the foot. A longitudinal section through the shaft and head of the left second phalanx is shown in Figure 3. The contour of the head was somewhat irregular, a bulge in the most distal part inferiorly being especially prominent. In one situation, indicated by the letter A, the dense connective tissue in contact with the bone contained cartilage cells. This part is shown under higher magnification in Figure 4 (not taken from the same section), in which the appearances are like those in Figure 13, case 2. These changes are looked upon as representing an early stage in the formation of osteochondromatous tumors (cf. Fig. 4 with Figs. 13 and 12, case 2). In Figure 3 there seemed to be a rather large amount of adipose tissue in the head of the second phalanx, but a comparison with similar sections from a normal bone of the same site, removed from the foot of an adult of the same age, showed no appreciable difference in the amount of adipose tissue in the head of the bone.

The soft tissue forming the prominence at the end of the left second toe (Fig. 1) was composed of adipose tissue and fibrous tissue; this was confirmed microscopically, and there were no special features except that fibrous tissue was especially conspicuous around some nerve bundles and occasionally within such bundles. As the large, soft tumor on the ball of the foot was continuous on the plantar aspect of the second toe with the tumor at the end of the second toe, it seemed likely that the tumor on the ball of the foot would have shown similar microscopic appearances if it had been available for histologic study. Tissue

from the plantar aspect of the proximal part of the left second toe, however, was available for study, and it showed conspicuous changes of neurofibromatosis which are illustrated in Figures 5 to 8. In Figure 5 the dark areas represent much altered nerve bundles, but the most altered nerve bundle, scarcely recognizable as such in this figure, is represented by a rather large area, in the center of which is the letter A. Cross sections of axis cylinders could be recognized clearly in this area. All of these altered nerve bundles were bound together by dense fibrous tissue. The nerve bundle indicated by the letter B is shown more highly magnified in Figure 6. It is suggested that the increased cellularity of the bundle was due largely to proliferation of neurilemmal elements. Figure 7 shows two abnormal nerve bundles (or parts of the one bundle), very cellular in their central parts but densely fibrous in their peripheral parts; this fibrous tissue merged in the fibrous bands which were separated by adipose tissue. The fibrous tissue and adipose tissue in Figure 7 are regarded as part of the neurofibrolipomatous condition which is thought to account for the increased bulk of the soft tissues of the second toe. In Figure 8 the nerve bundles proper differ very little from normal, but they are surrounded by dense fibrous tissue. Appearances like these might result from the formation of fibrous tissue in a chronic inflammatory reaction of long standing, but in the present case they are thought to be part of the general neurofibromatous condition and not inflammatory. Such an appearance is seen commonly in association with macrodactyly (cf. Fig. 8 with Fig. 14, case 2); a similar appearance is seen in Figure 15, case 3.

Changes akin to those affecting the nerves in this case are seen also affecting the blood vessels. The proportion of Figure 3 indicated by the letter B is shown more highly magnified in Figure 9. In Figure 9 the vascular channels have thick walls. Similar changes in blood vessels were noted in a case of ganglioneuroma of the alimentary tract reported by Poate and Inglis.[6]

In the part of Figure 9 indicated by letter A, small clusters of cells which are thought to be glomus cells were related to small vascular channels; overgrowth of fibrous tissue was conspicuous in this area.

In case 1 the outstanding abnormalities are neurofibromatosis affecting nerve bundles, associated with macrodactyly, neurofibrolipoma, osteochondromata, and changes in blood vessels.

Case 2

The patient (P.U.S. 129) was a male child. Notes made when he was 10 months old state that the left hand was deformed at birth and both breasts were greatly enlarged, especially the right. One finger had

been amputated previously. The tumor of the right breast was amputated at this time. The specimen appeared to the naked eye to be composed of fatty tissue under normal skin. This was confirmed by histologic examination, and the tumor was regarded as purely lipomatous.

When the child was 3½ years old he came under observation again. The notes made at this time state that the condition had been present since birth, and that the left thumb and index finger and the right breast had previously been removed (whether the right thumb was removed at the same time as the index finger is not clear from the notes). On this occasion the scar of the operation for removal of the fatty tumor of the right breast was evident. Left thumb and index finger were absent, the left fifth digit was deformed and greatly enlarged; the nail was small. The left forearm was much larger than the right. The appearance of the forearms and hands at this stage is shown in Figure 10. The left fifth digit and the tumor of the left breast were amputated.

The *tumor of the left breast* (after fixation) measured 12.5 by 8.5 by 3.5 cm. On section it was seen to be composed of adipose tissue presenting no special features; no mammary tissue could be seen in it. Microscopically, the portion embedded showed adipose tissue but no mammary tissue. The tumor was regarded as a lipoma.

The *left fifth digit* (after amputation) was examined radiologically and no bony abnormalities were evident. After fixation this digit in its largest part measured 2.3 cm. laterally and 2 cm. anteroposteriorly. The digit (including the phalanx) was cut through with a knife without any appreciable sense of resistance or grittiness. On cross section the enlargement of the finger appeared to be due to adipose tissue. Figure 11 shows a transverse section through the second phalanx; the preparation had not been decalcified. This is not the largest part of the finger; the largest part was proximal to this. The enlargement was due mainly to adipose tissue. The tendon (somewhat broken up) is seen close to the palmar aspect of the phalanx. The letter A indicates a broad area of cartilage (which was not revealed in the skiagram); a portion of this area is seen under higher magnification in Figure 12 which shows a broad zone of cartilage resting on trabeculae of bone and covered on the free surface by a zone of dense fibrous tissue. This lesion bears a close resemblance to that shown in Figure XI in the paper by Bell and Inglis.[3] In Figure 13, which represents a portion of the second phalanx a short distance from that illustrated in Figure 12, an earlier stage in the formation of an osteochondromatous tumor is seen; the appearances resemble those in Figure 4, case 1, but in Figure 13 the cartilage cells are even less clear than in Figure 4.

Vascular changes are not conspicuous in this case, nor indeed are changes in nerve trunks; Figure 14, however, shows some increase of fibrous tissue around nerve trunks, the appearances somewhat resembling those illustrated in Figure 8, case 1, and Figure 15, case 3.

It is strange that lipomata should have affected both mammary regions in case 2. It is thought, provisionally, that these masses of adipose tissue, like the fatty tissue in the enlarged little finger, may have been due to a local influence of neural intrinsic factor. An open mind is kept on this point because Adair, Pack, and Farrior [7] described a boy 11½ years old with Frölich's syndrome, well developed breasts, small penis and undescended testes, whose left hand and arm were considerably larger than the right, the thenar eminence being the size of a lemon; the thumb and first finger were likewise "much hypertrophied." It is suggested, as a result of the present study, that mammary enlargement in the case recorded by Adair, Pack, and Farrior may have had an endocrine basis, and have been a secondary effect, the enlargement of the limb and the endocrine disease being primary conditions due to a common underlying intrinsic factor (neural or basic).

Case 3

The patient (P.U.S. 6524), a young man, 19 years of age, had a tumor of the index finger of the left hand. The only disability was mechanical. The tumor was removed surgically, and during its removal the surgeon cut the superficial flexor tendon with which the tumor was closely related. The tumor was separate from the skin and not adherent to the bone. The patient had a similar tumor of the thumb on the same side. The specimen was approximately 6 cm. long, the larger end measuring 1.7 by 1.1 cm. in cross section, the smaller end 0.8 by 0.6 cm. The specimen had a somewhat irregular surface, and appeared to contain adipose tissue.

Adipose tissue was abundant, as is seen in Figure 16, but conspicuous changes were related to nerves and nerve endings. Figure 15 shows nerve bundles surrounded by dense fibrous tissue, the appearance resembling those shown in Figure 8, case 1, and in Figure 14, case 2. Enlarged and altered pacinian corpuscles were a conspicuous feature. Some appeared larger than normal, but apart from size their appearance was altered due to increase of fibrous tissue and blood vessels in them. Figure 16 shows one of the smaller pacinian corpuscles, less laminated than is normal because of increase of fibrous tissue; blood vessels were fairly conspicuous in this corpuscle, but not as much so as in many others in this specimen.

This case presents features relating to tactile corpuscles that call to

mind the case described by Cammermeyer.[8] It also has points in common with cases 1 and 2 in the present series and with the case described by Bell and Inglis,[3] all 3 of which were characterized by macrodactyly.

Discussion

Before considering local gigantism as such, two other features will be discussed, softening of bone in neurofibromatosis and osteochondromata associated with macrodactyly.

Softening of Bone

In case 2 in the present series a phalanx was cut through with a scalpel without difficulty even though the preparation had not been decalcified; although evidence in keeping with neurofibromatosis was present in the soft tissues of the finger, none was present in the softened bone. Similarly, no neurofibromatous tissue was found in the compressed vertebrae at the kyphotic angle of the patient with generalized neurofibromatosis described in an earlier paper (Inglis [2]). It was suggested in that case that the vertebral lesion was due to the influence of intrinsic factor and was linked with neurofibromatosis at the basic intrinsic factor level. It is now suggested that the softening of the phalanx in case 2 of the present paper is to be explained along similar lines.

Osteochondromata Occurring in Association with Macrodactyly

In cases 1 and 2 in the present paper, and in the case of macrodactyly reported by Bell and Inglis,[3] osteochondromata were present on the phalanges. Cartilage appears to be the main element in these tumors. In the early stages of development of the tumor, cartilage cells seem to make their appearance gradually in connective tissue in close relation to the surface of the bone, and the bone seems to take part in the formation of the tumor. The earliest recognizable stage in the formation of the tumor is illustrated in Figure 13, case 2; here the surface of the phalanx can be observed to be rough and irregular, and the slight bony overgrowth can be seen projecting into the adjacent connective tissue; the very early formation of cartilage cells in the connective tissue of the tumor is only just recognizable. A somewhat later stage in the formation of the tumor is illustrated in Figure 4, case 1. Individual cartilage cells are still difficult to recognize, but the fibrous tissue is now more homogeneous, and the general resemblance of the tumor to ordinary cartilage is closer. It is evident (especially on the left side of Fig. 4) that the lesion involves both the bone of the phalanx and the adjacent connective tissue. In Figure 12, case 2, the cartilage of the tumor bears a close resemblance to normal hyaline cartilage.

The interpretation placed upon these appearances is that the cartilage of the tumors arises not from misplaced foci of hyaline cartilage of the epiphyses, but as a result of a change in the fibrous tissue in immediate relation to the bone of the phalanx. In support of this opinion the results of the experiments of Fischer[9] are cited. Fischer stated that if a fragment of pure embryonic articular cartilage without the perichondrium is placed in a culture medium, no growth occurs, but if the perichondrium is included, or a whole piece of extremity is transplanted, the cartilage tissue begins to grow and may assume a considerable size. One gets the impression, said Fischer, that the new cartilage does not emanate from the original fragment, but is produced by a transformation of the fibroblasts.

Comment. Since the osteochondromatous tumors in cases 1 and 2, and in the case of macrodactyly described by Bell and Inglis,[3] were present in anatomical parts which in each case showed neural abnormality regarded as essentially the same as in neurofibromatosis, and since in Cammermeyer's[8] case of tumor of tactile organs of a finger there was an exostosis of the same finger, it is suggested that the presence of osteochondromatous tumors in a part where there is evidence of neurofibromatous involvement is not a mere coincidence but that both are due to the influence of neural intrinsic factor of neurofibromatosis (or of basic intrinsic factor).

Local Gigantism

Three suggestions have been offered to account for local enlargement of an extremity or part of an extremity: (a) abnormality in nerve supply, (b) abnormality in blood supply, (c) abnormality in hormonal control. Each of these will now be considered.

Abnormality in Nerve Supply

Evidence strongly suggests that local gigantism as exemplified by macrodactyly is related to neurofibromatosis. The case described by Bell and Inglis[3] showed "plexiform neuroma" of the median nerve leading to the enlarged part, and the histologic appearances in case 1 of the present series are strongly suggestive of neurofibroma. No evidence of generalized neurofibromatosis was present in these cases, but Moore[10] recorded examples of local gigantism with related neurofibroma in which there was spinal curvature (due to misshapen vertebrae) or widespread patches of pigmentation. Moore thought that endarteritis was present in lesions from his cases 3 and 4, but his illustrations do not appear to be consistent with that opinion. In a later paper, Moore[11] discussed macrodactyly and associated peripheral

nerve changes. In all 5 of his cases pathologic changes were found in the peripheral nerves. In one the changes were definitely classified as neurofibroma. In the other 4, the changes were an increase in endoneurial fibrous tissue, with evidence of degenerative changes in the nerve fibers; 3 of these 4 showed at least some of the clinical stigmata of neurofibromatosis. Moore said it is believed that the nervous system exerts some controlling action in the process of growth, and that the impaired nerves fail in this function, resulting in uncontrolled or uninhibited growth; but whether there are any changes in the central nervous system which result in the change in the peripheral nerves is not known.

Comment. In the present paper it is suggested that the increased growth of the enlarged digits in macrodactyly is influenced by the neural intrinsic factor of neurofibromatosis (or basic intrinsic factor) acting locally, and that it is not due to control by the nervous system in the ordinarily accepted sense, that is, by neurons and their axons. Bland-Sutton's [4] case, cited above, of plexiform neuroma of the musculospiral nerve and its branches without enlargement of digits of the affected limb is thought to be in keeping with this view.

Abnormality in Blood Supply

The common occurrence of cutaneous angiomata in patients suffering from neurofibromatosis and the frequent mingling of these two conditions in such patients is considered, as a result of the present study, not to be a coincidence, but to be due to a common underlying factor. Weber [12] drew attention to a group of cases in which hypertrophy of one limb, or else hemi-hypertrophy, is found to be associated with tumor-like overgrowth in the corresponding portion of the vascular system. It seemed, he said, as if the hypertrophy, affecting the soft tissues, and usually the bones as well, depended in these cases, to some extent at least, on excessive vascularity of the affected parts caused by the overgrowth of the blood vessels. In a later paper, Weber [13] referred to the condition as hemangiectatic hypertrophy of limbs.

Bell and Inglis [14] described a hemangioma of the right leg of a girl, 17 years of age. Two pigmented, slightly raised areas were present on the right leg, one over and one immediately below the medial malleolus. The right leg had gradually increased in size but it was only 33 cm. in length as compared with 36 cm. for the normal (left) leg. Examination of the amputated limb showed an angioma extending from the ankle almost to the knee posteriorly. The large vascular sponge-like tissue permeated and to a large extent replaced the muscles. This case corresponds with Weber's description so far as the angiomatous growth is concerned, but though the girth of the affected leg was greater than

that of the normal leg, the affected leg, despite the great vascularity, was shorter than the normal leg. It would seem, therefore, that excessive vascularity does not of itself cause elongation of the affected limb. Although no special significance was attached, at the time, to the two pigmented areas of the skin of the affected leg, it is now thought that they are in keeping with the view that neural intrinsic factor, or basic intrinsic factor, predisposed to the developmental vascular abnormality.

Harris and Wright[15] investigated clinically a case of so-called hemangiectatic hypertrophy of a limb of a boy, 10 years of age. They said that experiments on kittens, which they carried out, indicated quite clearly that the destruction of the sympathetic innervation to the forelimb, with its resultant increased blood supply, caused no increase in the length of its bony structures.

Comment. It is suggested that when there is elongation of a limb associated with extensive hemangioma in the affected part, the elongation of the limb is not caused by the excessive vascularity, but that the hemangioma and the elongated overgrowth of the limb have a common underlying factor, namely, neural intrinsic factor or basic intrinsic factor.

Abnormality of Hormonal Control

The hormone in question is the hormone of the anterior lobe of the pituitary gland, and the problem now to be considered is the relation of local enlargement of a part to the general enlargement found in gigantism and in acromegaly.

Local Gigantism

Marie,[16] in his original thesis on 2 cases of acromegaly, said of his case 2 that the toes were very much larger than those of an ordinary person, especially the great toe, the end of which was a little clubbed.

Marie and Marinesco[5] made a study of the pathologic anatomy of acromegaly. They said that the peripheral nervous system shows lesions only in the regions affected by the acromegalic process. They chose for illustration sections of the great toe of an acromegalic patient (whether the great toe of the original thesis, of which the end was a little clubbed, is not stated). In their Figure 5 there are fibrous changes around nerve bundles which it seems possible may have been akin to the changes illustrated in my Figure 8, case 1. Figure 11, case 2, in the present paper, except for the cartilaginous tumor, resembles Figure 6 in the paper by Marie and Marinesco, and Figure 14, case 2, of the present paper somewhat resembles Figure 5 in the paper by Marie and Marinesco. Those authors regarded the changes shown in their Figure 5 as due to perineuritis and endoneuritis, but, in view of the appearances

shown in my Figure 8 of case 1, Figure 14 of case 2, and Figure 15 of case 3, it seems possible that the perineural fibrosis in the great toe described by Marie and Marinesco was part of a neurofibromatous state and not inflammatory. In the toe examined by Marie and Marinesco the enlargement was regarded as part of the acromegalic process, but as a result of the present study it is suggested that in local (as distinct from general) enlargement of digits in acromegaly the enlargement may be due not to hormone from the anterior lobe of the pituitary gland, but to neural intrinsic factor of neurofibromatosis.

Zondek,[17] under the heading partial acromegaly, described a girl, 17 years of age, whose gums in both jaws were thickened, hard, and showed irregular proliferations; the lips were coarse, and small warts were noticed at the tip and sides of the tongue. Histologic examination of the gums and the warts on the tongue revealed nothing but fibrous proliferation. It is suggested that the condition described by Zondek corresponds to the hypertrophy of the gums (with general dwarfism) described by Hutchinson,[18] the gingival hypertrophy (with marked hypertrichosis of the scalp) described by Thoma,[19] and the hypertrophy of the gums in a patient with facial hemihypertrophy and neurofibromatosis described by Brain.[20] It is suggested that all of these examples of hypertrophy of the gums are manifestations of neurofibromatosis and are not due to anterior pituitary hormone.

Zondek,[17] discussing localized gigantism, said that, as with acromegaly, the distinction must be made between generalized and localized gigantism. He said that Guenther[21] described a girl, 10 years of age, with enormous localized gigantism of the right leg and the fourth and fifth fingers of the left hand. She also showed congenital lipomata on the back, the nape of the neck, and the right lumbar region; on the left side of the neck, the shoulder and the forearm there were numerous hard nodules ranging in size from a hempseed to a bean as well as several large lipomata. In other cases, said Zondek, generalized and localized gigantism can be combined. He referred to a girl of 11 years, unusually tall for her age and affected with very considerable gigantism of the right leg and of the first and second toes of both feet. On the left side of the thorax there were two lipomata. The abnormal size of the foot had been noticed at birth. One aunt had polydactyly, the other had multiple lipomata. It is suggested, as a result of the present study, that these examples of partial gigantism mentioned by Zondek, as well as associated conditions such as lipomata, were due to the influence of neural intrinsic factor of neurofibromatosis (or basic intrinsic factor) acting locally, and not to anterior pituitary hormone.

Bauer[22] said that a twin child observed by Allaria[23] had partial

gigantism of three fingers of his right hand; his mother was acromegalic and had a goiter. It is interesting to note that in that family the local gigantism of digits was present in the child, the acromegaly in the mother, whereas in the acromegalic woman, 36 years of age, described by Sternberg,[24] both conditions appear in the photograph to have been present in one individual. In Sternberg's Figure 3 the hands and the right foot of the woman seem uniformly enlarged, but, though not commented on by Sternberg, on the left foot the four outer toes and corresponding part of the foot seem enlarged but relatively little, and the great toe and corresponding part of the foot appear greatly enlarged and resemble the enlarged digits of macrodactyly.

Concomitant Lesions in Acromegaly

Neurofibromatosis

It is suggested that in Sternberg's case,[24] the acromegaly, presumably due to disease of the anterior lobe of the pituitary gland, accounted for the general enlargement of the acral parts, but that neural intrinsic factor of neurofibromatosis influenced the greater local enlargement of the left great toe and inner part of the left foot. It is further suggested that neural intrinsic factor, or basic intrinsic factor, influenced the development of the pituitary body, so that the pituitary abnormality and the local enlargement of the great toe and corresponding part of the left foot were both of the same order, the general enlargement of the acral parts being secondary to the lesion of the anterior lobe of the pituitary gland.

The suggestion that neural intrinsic factor, or basic intrinsic factor, may influence the abnormal development of the anterior pituitary gland in acromegaly raises the question of the association with acromegaly of other lesions of the neurofibromatous complex besides macrodactyly or local gigantism. It is suggested that there is a resemblance between the enlargement of the peripheral and sympathetic nerves in the acromegalic patient whose body was examined after death by Henrot[25] (as recorded by Marie[16]), and the diffuse neurofibromatosis involving the cranial, peripheral, and sympathetic nerves, accompanied by a tumor of the hypothalamus, in the case reported by Aegerter and Smith.[26] Cases are on record in which acromegaly and von Recklinghausen's neurofibromatosis have been present in the same patient; those reported by Ormond[27] and by Freund[28] are examples of this association. If, however, the lesions of neurofibromatosis are inconspicuous, their possible significance may be overlooked.

It may be mentioned that in all 4 cases of acromegaly in the series of Cushing and Davidoff,[29] those authors mentioned (without comment)

signs which, taken together, may have etiologic significance. Their case 1 showed a large hirsute patch on the sacral region; their case 2 showed some fibromata mollusca over the back and chest and a small lipoma below the scapula, as well as "bull-dog scalp"; their case 3 showed a few areas of pigmentation, numerous fibromata mollusca scattered over the shoulders and back, and a lobulated lipoma weighing 150 gm. in the right inguinal region; their case 4 showed many pigmented moles, particularly in the lower thorax, and several fibromata mollusca.

Cutis Verticis Gyrata ("Bull-Dog Scalp")

It is suggested that the "bull-dog scalp" in case 2 of Cushing and Davidoff[29] is akin to the neurofibromatous or allied lesions occurring in their 4 cases. Further, the coarseness of the hair (although there was practically no beard) of the "bull-dog scalp" of this case of acromegaly has points in common with hairy patches related to nevi of the skin, hairy tufts overlying spina bifida occulta (Bland-Sutton[4]), and the coarse hair of scalps showing cutis verticis gyrata in idiots (Tredgold[30]), in whom, in keeping with the present hypothesis, a common factor may be responsible for the abnormalities of both cerebrum and scalp. It is suggested that all examples mentioned above have a common basis and are predisposed to by neural intrinsic factor or basic intrinsic factor.

Disproportionate Splanchnomegaly

Cushing and Davidoff[29] said that extraordinary splanchnomegaly characterizes most cases of acromegaly and is often out of all proportion to the size of an acromegalic giant. It will be seen from their case reports that the splanchnomegaly is disproportionate not only to the general enlargement of the body, but disproportionate and variable as between different organs of the body. For example in their case 4, although there was moderate splanchnomegaly of liver, kidneys, and spleen, the gastrointestinal tract was normal throughout; whereas in their case 1 with visceral splanchnomegaly, the whole alimentary tract was enlarged, the stomach being enormous. Cushing and Davidoff said "there is unquestionably some underlying law that affects the degree of splanchnomegaly which we do not yet clearly understand." They also said that Marie's attribution of the enlargement to an increase of connective tissue can hardly be sustained, to judge from their own cases in which the huge organs on the whole were histologically well within normal limits. It is recognized that, apart from their general effects, the hormones of ductless glands may bring about local changes of growth and development, especially in relation to secondary sex characters. It is therefore understandable that, when hyperplasia or neoplasia of

the anterior lobe of the pituitary gland is present in a patient, enlargement of organs or parts in that patient should be regarded as due to the secretion of the anterior lobe of the pituitary gland. Evidence in support of the opinion that neural intrinsic factor influences the development of partial gigantism of the extremities has already been presented. Could disproportionate splanchnomegaly be due to the influence of neural intrinsic factor or basic intrinsic factor?

Batchelor and Maun[31] mentioned hepatomegaly, associated and unassociated with splenomegaly, as occurring in patients with glycogenic tumors of the heart. In an earlier paper (Inglis[1]) it has been suggested that neural intrinsic factor or basic intrinsic factor may influence the development of these cardiac tumors. Straus, Merliss, and Reiser[32] referred to 2 instances of enlargement of liver and spleen in gargoylism in which microscopic examination revealed no lipoidosis. It is suggested, as part of the present hypothesis, that intrinsic factors may influence the development of gargoylism.

The possible bearing that abnormal innervation may have in the disproportionate splanchnomegaly of acromegalic patients seems deserving of consideration in view of the experiments of Timme.[33] He ligated the vagus nerve a short distance above the diaphragm in cats which were killed between 111 and 140 days after operation. The ligation was carried out in such a way as to leave a sufficient number of fibers to carry on some of the vagus function. Timme found that the stomachs of the cats whose vagi were tied became appreciably larger and had thicker walls than the stomachs of normal controls. In the colon there was produced an increase in both length and caliber, the changes, Timme thought, corresponding to those in Hirschsprung's disease. In this regard it is interesting to note that Preiser and Davenport[34] recorded Hirschsprung's disease in the sibling of a patient with neurofibromatosis.

Enlargement of Thymus and of Lymphoid Tissue

Marie and Marinesco[5] said that Klebs[35] was struck by the existence of the thymus in his case of acromegaly. Sternberg[24] found that in acromegaly the thymus is frequently preserved at full size. Cushing and Davidoff[29] said a persistent thymus is a common post-mortem finding in acromegaly; it was present in 2 of their 4 cases. Hyperplasia of the thymus and of lymphoid tissue, however, is absent in many cases of acromegaly and it may occur in association with other conditions. For example, it was present in the case of Turner's syndrome, described by Atria, Sanz, and Donoso.[36] In the report of this case it is recorded

that the pituitary gland showed no special change. It would seem, therefore, that enlargement of the thymus and of lymphoid tissue may occur apart from disease of the anterior lobe of the pituitary gland, and that increased anterior pituitary secretion is not then its cause. It is suggested, in keeping with the present hypothesis, that when enlargement of the thymus is found in a patient with acromegaly, a link between the pituitary and thymic lesions may exist at the basic intrinsic factor level.

Involvement of Other Hormonal Glands

Cushing and Davidoff[29] wrote that one might almost speak of acromegaly as a disease characterized by a pluriglandular tendency to adenomatous formations; they regarded the changes in the pituitary gland as primary, those in the other hormonal glands as secondary. Murray,[37] on the other hand, described 2 cases of acromegaly: one with a large goiter, the other with exophthalmic goiter. In both cases the first change which took place was an enlargement of the thyroid gland. Murray said that the co-existence of the two diseases suggests that there may be some common cause. In keeping with Murray's view is the opinion formed as a result of the present study, namely, that while recognizing the influence that one hormonal gland may have on others, when two or more hormonal glands are abnormal the abnormality may be due to influences acting locally on each gland, and, on this hypothesis, the suggestion is made that in Murray's case basic intrinsic factor may have influenced the development of both thyroid and pituitary abnormalities.

Syringomyelia

Holschewnikoff[38] described syringomyelia and acromegaly in the same patient; the autopsy on the body of this patient was performed by von Recklinghausen.[39] Petrén[40] also described the simultaneous occurrence of acromegaly and syringomyelia; he considered that acromegaly may be hereditary and stated that 2 siblings of his case 1 also had acromegaly. Macbride[41] noted that the association of acromegaly and syringomyelia in the same patient has been mentioned in the literature for many years and has now become accepted as more than a coincidence; he described 2 such cases (clinical). It is suggested, as a result of the present study, that when syringomyelia and acromegaly occur in the same patient, one has not led to the other, but intrinsic factor (probably at the basic intrinsic factor level) has influenced the development of both.

Summary and Conclusions

Local softening of the bone, such as was present in the phalanx of case 2 in the present paper, is due to the influence of the neural intrinsic factor of neurofibromatosis or of a basic intrinsic factor.

Osteochondromata of the phalanges associated with macrodactyly are due to the influence of neural intrinsic factor of neurofibromatosis or of basic intrinsic factor.

Local gigantism is due to the influence of neural intrinsic factor of neurofibromatosis (or basic intrinsic factor) acting locally, and is not due to faulty control by the nervous system in the ordinarily accepted sense, that is by neurons and their axons.

When elongation of a limb is associated with extensive hemangioma in the affected part, the elongation of the limb is not caused by excessive vascularity and increased blood supply, but the hemangioma and the elongated overgrowth have a common underlying factor, namely, neural intrinsic factor (or basic intrinsic factor).

When local gigantism occurs in a patient with general gigantism or with acromegaly, the local enlargement is due to neural intrinsic factor of neurofibromatosis, the general enlargement being due to the hormone of the anterior lobe of the pituitary gland.

The lesion of the pituitary gland responsible for general gigantism or for acromegaly is predisposed to by the influence of intrinsic factor, probably at the basic intrinsic factor level.

Concomitant lesions of acromegaly such as local gigantism, cutis verticis gyrata, syringomyelia, lipomata, and neurofibromatosis are not due to the hormone of the anterior lobe of the pituitary gland, but are of the same order as the pituitary lesion; intrinsic factor (neural or basic) underlies them all, including the pituitary lesion.

I wish to thank Drs. E. M. Humphery, R. D. K. Reye, and A. L. Webb for specimens and notes, Mr. L. Findlayson for technical assistance, and Mr. S. Woodward-Smith and Mr. K. Clifford for taking the photomicrographs.

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DESCRIPTION OF PLATES

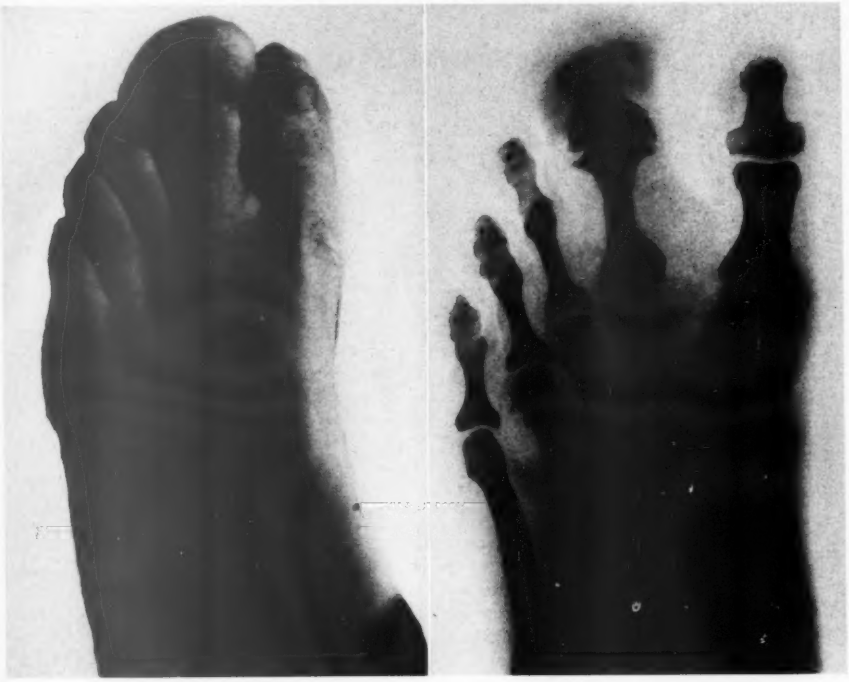
PLATE 151

FIG. 1. Case 1. Appearance of the left foot when the patient was 24 years of age.

FIG. 2. Case 1. Skiagram of the left foot taken at about the same time.

FIG. 3. Case 1. A longitudinal section through the shaft and head of the second phalanx of the left second toe. The part indicated by the letter A is shown more highly magnified in Figure 4, and the part indicated by the letter B is shown more highly magnified in Figure 9. × 3.

FIG. 4. Case 1. The part of Figure 3 indicated by the letter A is here shown more highly magnified (Figs. 3 and 4 were not taken from the same section, but from sections close to one another in the series). An osteochondromatous tumor at an early stage of development is seen; cf. Figures 13 and 12 (case 2). × 50.



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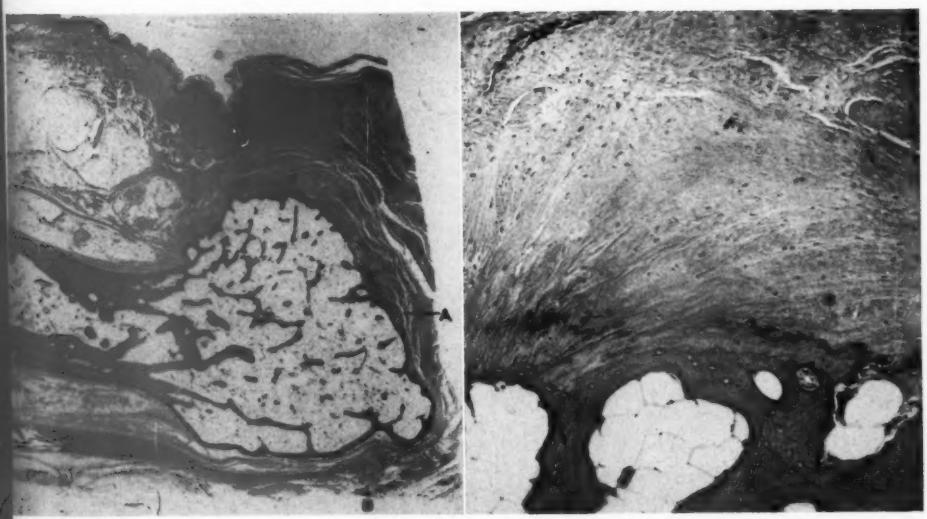


3 4

Inglis Local Gigantism

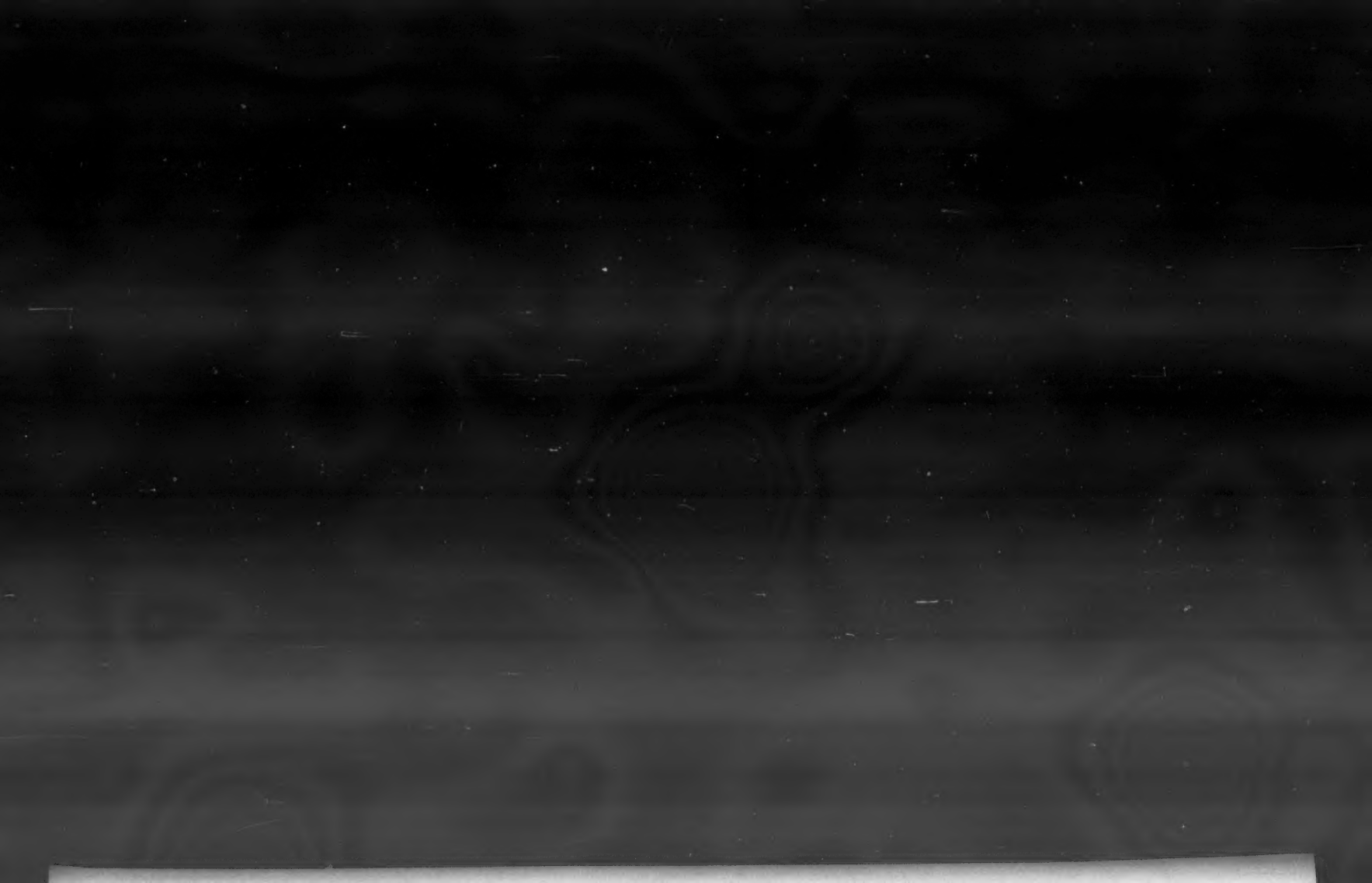
PLATE 152

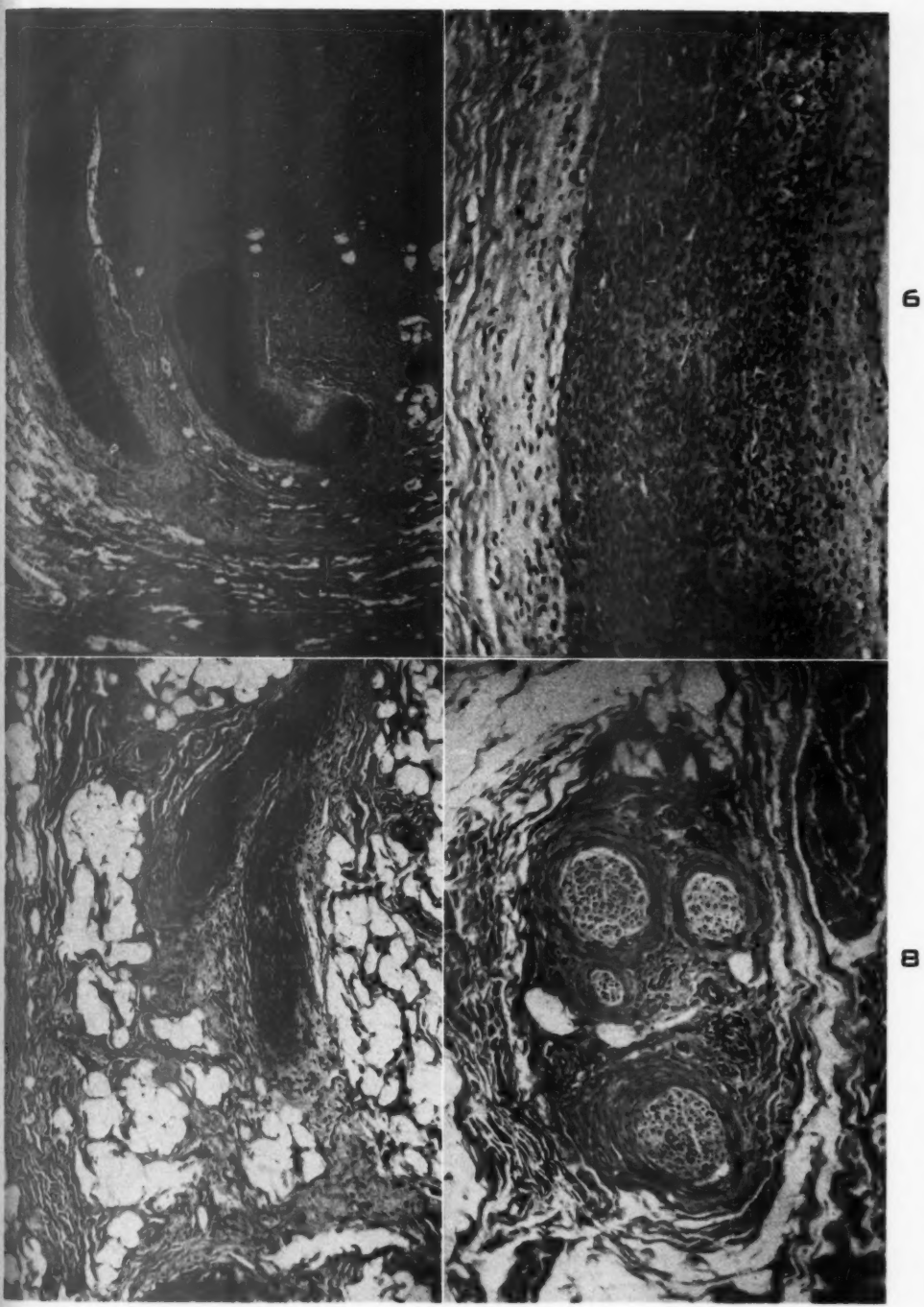
FIG. 5. Case 1. Tissue from the plantar aspect of the proximal part of the left second toe. The dark areas represent much altered nerve bundles, the one indicated by the letter B is shown more highly magnified in Figure 6. The letter A is situated in the center of a section of a greatly enlarged and altered nerve bundle which can scarcely be recognized as such. The condition is thought to be neurofibromatous. × 25.

FIG. 6. Case 1. The part of Figure 5 indicated by the letter B is here shown more highly magnified. It is suggested that the increased cellularity of the nerve bundle is largely due to proliferation of neurilemmal elements. × 100.

FIG. 7. Case 1. Soft tissue from the left second toe. Two abnormal nerve bundles (or parts of one bundle) are present. They are very cellular in their central parts, but densely fibrous in their periphery. The cellularity of the central parts is thought to be due (at least in part) to neurilemmal proliferation. The fibrous tissue of the peripheral parts merges in the fibrous bands which are separated by adipose tissue. The fibrous tissue and adipose tissue shown in this figure are regarded as part of the neurofibrolipomatous condition which is thought to account for the increased bulk of the soft tissue of the second toe. × 50.

FIG. 8. Case 1. Soft tissue from the left second toe. The nerve bundles proper differ little from normal, but they are surrounded by dense fibrous tissue which is regarded as part of the neurofibromatous process. Cf. Figure 14 (case 2) and Figure 15 (case 3). × 100.





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8

Inglis Local Gigantism

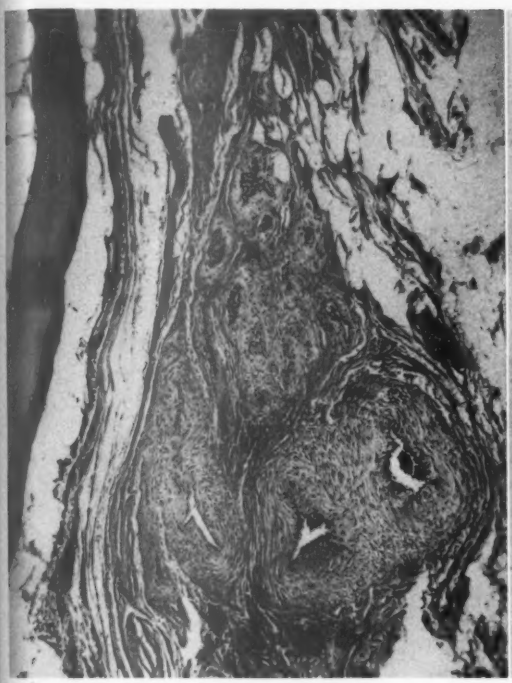
PLATE 153

FIG. 9. Case 1. The part of Figure 3 indicated by the letter B is here shown more highly magnified. An outstanding feature is the increase in thickness of the walls of the vascular channels. In the part of Figure 9 indicated by the letter A, small clusters of cells, which are thought to be glomus cells, are related to small vascular channels. × 50.

FIG. 10. Case 2. The forearms and hands of the patient at 3½ years of age. The left fifth digit is deformed and greatly enlarged; the nail is small. The left forearm is much larger than the right.

FIG. 11. Case 2. A transverse section through the second phalanx of the left fifth digit. The enlargement of the digit is due mainly to adipose tissue. The tendon (somewhat broken up) is seen close to the palmar aspect of the phalanx. The letter A indicates a broad area of cartilage; a portion of this area is shown under higher magnification in Figure 12. × 4.

FIG. 12. Case 2. The part of Figure 11 indicated by the letter A is here shown more highly magnified. The cartilage rests on trabeculae of bone, and is covered on the free surface by dense fibrous tissue. × 42.



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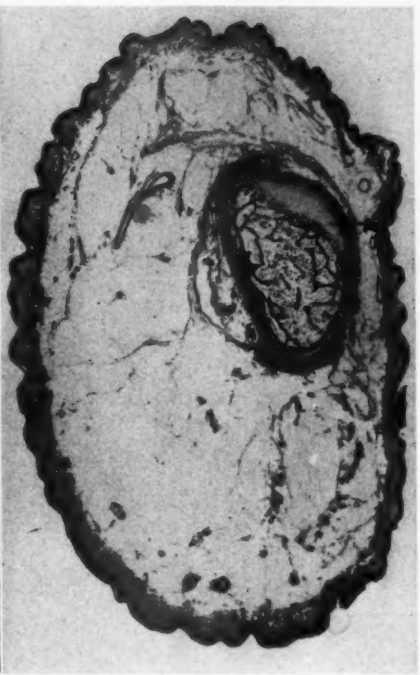

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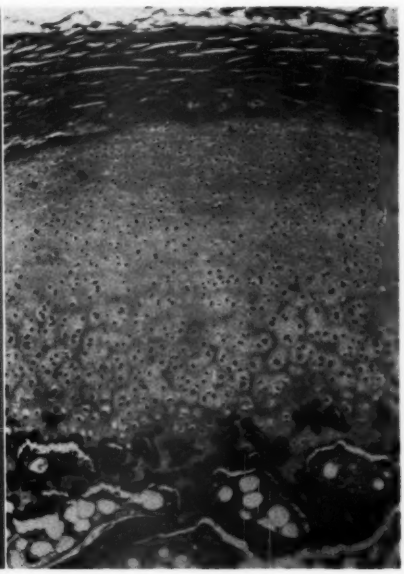


12

Inglis

Local Gigantism

PLATE 154

FIG. 13. Case 2. A portion of the second phalanx of the left fifth digit a short distance from the part illustrated in Figure 12. A very early stage in the formation of an osteochondromatous tumor is seen; compare the somewhat later stage evident in Figure 4 (case 1). × 91.

FIG. 14. Case 2. Portion of the soft tissue of the left fifth digit. There is some increase of fibrous tissue around nerve bundles; cf. Figure 8 (case 1) and Figure 15 (case 3). × 91.

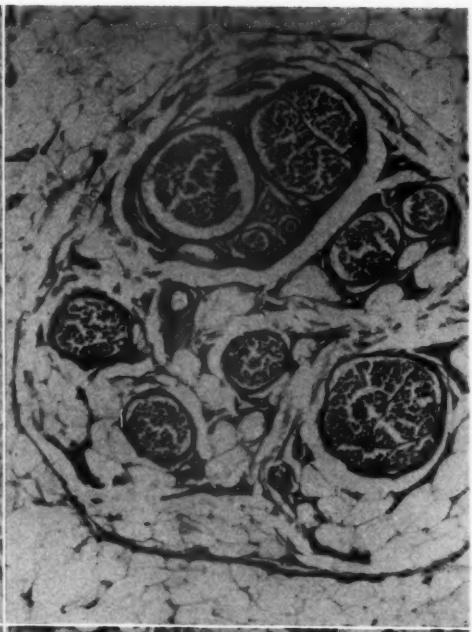
FIG. 15. Case 3. Portion of tumor of left index finger. Nerve bundles are seen surrounded by dense fibrous tissue; cf. Figure 8 (case 1) and Figure 14 (case 2). × 91.

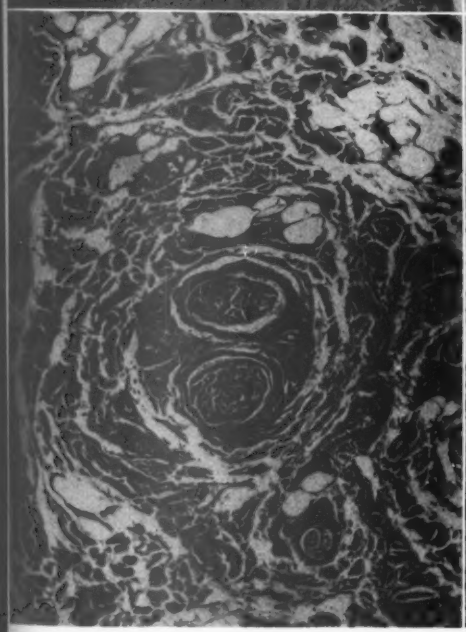
FIG. 16. Case 3. A portion of the tumor of the left index finger. Adipose tissue is conspicuous, and a somewhat abnormal pacinian corpuscle is included also. × 91.

A

13

14





15

16

Inglis Local Gigantism

THE ETIOLOGY OF LATERAL NASAL CLEFTS *

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In considering teratologic conditions, special interest is attracted to the moment of origin, to the manner of formation (formal genesis), and, finally, to the etiology (causal genesis). It is regrettable that even today very little is known about the etiology of malformations. This is true of the very rare lateral nasal cleft. Moreover, its bearers are nearly always viable so that a thorough examination of the whole organism is lacking for nearly all cases. Such an examination is necessary to answer the question whether the lateral cleft is a simple local malformation, with the remainder of the body perfectly normal, or whether other malformations, possibly of several internal organs, are present simultaneously. It is clear that every case which has been thoroughly examined, grossly and microscopically, in respect to each organ, becomes of importance for the elucidation of malformations in general, as well as of the lateral nasal cleft.

I am able to report a case of lateral nasal cleft with a complete necropsy and to illustrate certain gross and microscopic features. A stillborn female fetus, 27 cm. long, was sent by the Women's Clinic at Wels, Austria, to the Institute of Forensic Medicine at Innsbruck, by the Director of which I was asked to undertake an examination in order to determine, if possible, the cause of the nasal cleft. The mother was a primipara, herself perfectly healthy and coming from a family with no history of malformations. Her pregnancy had hitherto been perfectly normal. Two days after a profuse uterine hemorrhage with some pain, she was hospitalized and treated conservatively with sedatives and vitamin E. The fetus and an intact placenta were expelled 3 days later. Convalescence was afebrile and the patient was discharged 6 days later.

In Figure 1 is shown the malformed fetus of 5 lunar months' development. As seen from the right side, one is immediately struck by the serious disfigurement of the right ala nasi, which showed an upwardly turned cleft with a tissue defect. As appears more clearly in Figure 2, the nose was displaced toward the left, the tip deviating 4 mm. from the mid-sagittal plane. In consequence, the right lateral portion of the nose, as compared to the normal left part, appeared very much broad-

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† Dr. Stupka died February 14, 1950.

ened and flattened. Moreover, the malformed right ala nasi was three times as long as the normal structure on the left. The cleft of the right ala nasi was turned obliquely upward, and measured about 3 by 2 mm. The edges of the cleft were ridged and, especially on the medial side, appeared to have a cicatricial character.

In an enlarged view of the cleft (Fig. 3), a club-shaped appendix can be seen on the ala nasi. This was directed upward and had a blunt end. Its area of attachment was close to the caudal end of the lateral part of the cleft. The lighter-appearing area ventral to this is a portion of the nasal septum protruding into the cleft.

Serial sections were made through both sides of the nose in an approximately frontal direction. Figures 4 and 5 correspond to the ninth and eighth sections of the series. Microscopically, the projecting tag of tissue was found to consist of firm connective tissue, resembling fibrous callus, without apparent epithelial covering. It was practically avascular, infiltrated by inflammatory cells in its outer aspect, and, unlike the ala nasi, was completely free from hairs. The foot of this appendix was implanted in the ala nasi and in the area of implantation there were neither hairs nor sebaceous glands. A second peg-shaped appendage, composed of similar tissue, was attached to the inner surface of the cranial part of the ala nasi. Both of these formations are believed to be remnants of amniotic threads.

It may be assumed that, by abnormal processes during the excavation of the amniotic cavity or in consequence of inflammation of the amnion itself, local adhesion and fusion of the head-fold of the amnion to the nasal area took place. Later, at the fourth or fifth week, when accumulation of the liquor amnii separates the body surface from the amnion, one or several amniotic threads resulted from the primary adhesion. These threads, inserted at the previously well formed ala nasi, divided it and the cleft resulted, but later the threads themselves were torn by the withdrawal of the amnion from the surface of the fetus. Remnants of these threads were left on the specimen to mark their points of insertion.

Further evidence of this traumatic division may be derived from microscopic examination of nearby sections of the series through the defect. In the recessus apicis nasi the lateral part of the alar cartilage was lacking; the portion present was abruptly interrupted and was exposed entirely naked. The covering mucous membrane in this area and on the septum was swollen, almost entirely lacking in epithelium, infiltrated by inflammatory cells, and, particularly in the septum, richly supplied with strongly congested vessels, some of which ran parallel to

the surface but more were directed vertically in respect to the surface. Obviously this was a region of chronic inflammation. Since no bacteria were found, the probability that mechanical trauma was the chief etiologic factor receives added support.

The tension of the amniotic threads, possibly aided by pressure in a retroflexed uterus, was competent not only to cause division of the ala nasi by strangulation but also to produce the severe deflection of the entire nose to the left and broadening of the malformed right ala nasi (right:left::10 mm.:3 mm.). Further, a curvature of the cartilage of the lateral nasal wall on the right side was produced. This can be followed in subsequent sections of the series. The caudal end of the cartilage was shortened and surrounded by dense connective tissue, like scar tissue. The effect of the amniogenic trauma continued to be recognizable in sections lying more dorsally and consisted in straightening of the lumen and in the peculiar curvature of the cartilago nasi lateralis on the malformed side. This can be seen in Figure 6 which corresponds to section 27 of the series.

Further dorsally, the right side of the nose became entirely normal. Figure 7, prepared from section 49, shows approximate bilateral symmetry, with normal development for the fetal age, of all structures proper to this plane. Examination of additional serial sections, extending to the region of the clivus, showed the remainder of the nasal tract to be of normal conformation, with four turbinate bones on each side, a deep semilunar hiatus and well formed foramina and fila olfactoria. The vomer, posterior nares, and os palatinum were of normal conformation, the auditory canals and their cartilages were normal, the uvula was simple, and all further structures, including the cavernous sinuses, trigeminal nerves, and hypophysis, were properly developed.

An exhaustive post-mortem examination (A. Priesel, Vienna), both gross and microscopic, established that all viscera were normally formed and normally placed. The only essential difference from normal conditions was in the presence of extensive hemorrhages in the soft tissues of the cranium, the cranial cavity, abdominal cavity, cardiac atrial walls, and about the left adrenal gland. There were also bloody extravasations in the lateral ventricles and in the subdural space about the cerebellum. The corpus striatum and the thalamic areas showed only slight hemorrhage. Series of sections through the larynx and pharynx, trachea and thyroid gland, both eyes, and both ears were examined. All of these organs showed an adequate level of development and were normal except for general venous engorgement and scattered hemorrhages. Both congestion and hemorrhage must be considered consequences of the abrupt

interruption of pregnancy and rather sudden death and expulsion of the fetus.

From study of this case no difficulty arose in determining the etiology of the lateral nasal cleft. The remnants of amniotic threads on the defective edge of the ala nasi, the broadening of the injured ala nasi, and the extreme deviation of the entire nose to the opposite side, require an external, rather prolonged, and locally limited cause. Only amniotic trauma supplies an adequate explanation. Indirectly, this opinion is supported by the fact that all internal organs were found to be normally formed and without inflammatory or retrogressive changes to indicate any toxic or bacterial agent.

The lateral nasal cleft is a very rare malformation. Up to 1938 only about 20 cases were known and since then no further observations have been recorded in the abstract journals and reviews to which I have had access. The cases published previously were collected by me[1] in 1938. These can be grouped as slight, serious, and monstrous forms. The first affect only the ala nasi and are of variable extent. The others are always combined with additional clefts and other malformations, and, in the more extreme forms, cleft formation extends into neighboring regions, perhaps with prevention of the proper obliteration of the primary furrows of the face, or with disturbance of the orderly formation of the nasal sac and its connection with the region of the pharynx. Thus we find cases with homolateral fistula of the lachrymal sac, coloboma of the lower eyelid, and complete osseous atresia of the posterior nares (Stütz[2]), and others with harelip, an oblique facial cleft, or a medium nasal cleft.

The lateral nasal cleft involves the right and left sides equally. Two bilateral examples have been reported (Binnie[3] and Tsakyroglous[4]). The cleft is usually triangular with upwardly directed apex, usually starts in the mid-portion of the ala nasi, reaches a variable height, and the resulting loss of tissue is of variable extent. The slightest degrees, or "cleft equivalents," are represented by cicatrization (intra-uterine healed nasal cleft in the case of Lähr[5]), by congenital fibroma of the ala nasi (Ruttin[6]), or by a small cleft with upward displacement of tissue elements. This was true of the second case of Frangenheim,[7] which concerned a newborn girl with a pea-sized tumor on the lateral wall of the nose above the cleft. There was cartilage in the center of the tumor. There was a notch in the nasal bone of the malformed side and the lateral nasal cartilage was insufficiently developed. These cases and others strengthen the plausibility of the view that amniogenic trauma

may derange the proper development of the ala nasi to varying extent.

A teratoma (bidermoma) on a girl of 18 years with harelip and cleft palate, described by Salzer[8] in 1886, had the size of an ostrich egg. It was believed to have arisen from a lateral nasal cleft, was attached above the right ala nasi and penetrated the right frontal sinus. Salzer suggested that due to its close contiguity, the amnion had been embedded in the embryonic facial furrows and fused with the tissues of the head. Since the proamnion receives its mesodermic stratum during the period when the embryonic facial furrows are present, some mesodermic elements of the amniotic fold might have been detached during the fusion and subsequent separation. From these the bidermoma could have arisen.

The case of Glaus,[9] the only previous one with histologic examination, concerned an immature, newborn male infant with a reddish blue, plum-shaped "polypus" hanging from the upper angle of a triangular defect of the right ala nasi. This mass showed myxomatous, cavernous, and fibromatous tissues. Of the nasal bone of the same side only a small remnant was palpable, the remainder of the nasal skeleton and also the nasal cavity being normal. Glaus assumed a detachment of tissue in the region of the lateral nasal process, which is difficult to imagine from the mechanical side unless the process was amniogenic. However, multiple serious malformations were found. These involved the cardiovascular apparatus and limbs, and there were, as well, atresia of the esophagus with a tracheo-esophageal fistula, cystic kidneys, and a Meckel's diverticulum.

The possibilities for the origin of malformations fall into two main groups. The first group includes both the original, *i.e.*, hereditary, alteration of one or several genes in the ovum or sperm cell or both, and the dysmutation of genes before, during, or after copulation, but before amphimixis, *i.e.*, blastophthoria, through some extrinsic injurious agent. In the latter case, one might expect more serious and more numerous malformations, provided a fetus would develop at all. There is as yet no evidence that a lateral nasal cleft is due to heredity or blastophthoria.

The second main group of malformations consists of those in which the germ plasm was normal and at first developed in a normal manner, only to be subjected at various moments in intra-uterine life to injuries of various kinds, mechanical, toxic, radioactive, or infectious. Mechanically produced malformations will be chiefly exterior and may be solitary or multiple. Malformations of toxic or infectious cause may be expected to appear in various organs, the simultaneous involvement being explained by the fact that the organs concerned all happened to be in a

stage of great metabolic activity when they are particularly vulnerable to such injury (law of Child[10]).

When the reported examples of lateral nasal cleft are examined, they can all be assigned to the second of the main groups which have been outlined. None can be proved to be due to heredity, but all seem to have been due to injury of a primarily normal ovum or embryo during intra-uterine life. All cases hitherto reported support the conclusion that amniotic bands are the main cause of this rare malformation. This view is valid for the case of Glaus[9] in so far as the nasal cleft is concerned, although the other malformations present in his case may be properly charged to some noxious influence which simultaneously injured the primordia of several organs.

The amniotic genesis of the lateral cleft is particularly clear in the case which has been the subject of this report. A detailed investigation established that all internal organs were free from developmental faults and that the malformation of the nose was a solitary deviation from normal in this fetus. Amniotic remnants at the lateral part of the cleft completed the proof.

In addition to the theoretical considerations involved, two important practical points may be emphasized. Parents and patients may be advised that there is no evidence that lateral nasal cleft is a hereditary condition. The physician confronted with a viable bearer of a lateral nasal cleft may be assured, with a high degree of probability, that his patient is free from other important malformations. This may facilitate making a decision in favor of corrective surgical procedures.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 155

FIG. 1. Oblique view of fetus of 5 months' development, showing the cleft of the right ala nasi.

FIG. 2. The same lateral nasal cleft seen in full face view.

FIG. 3. An enlarged photograph of the lateral nasal cleft to show the appendages believed to be amniotic remains.

FIG. 4. A low power view of the ninth serial section through the defect. The projecting tags of tissue are interpreted as remnants of amniotic adhesions.

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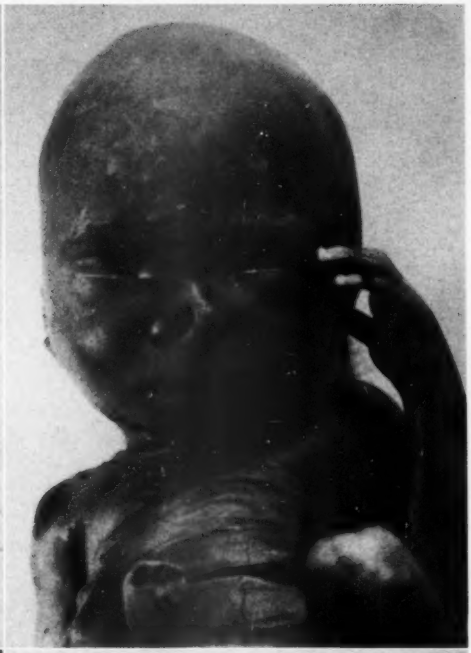

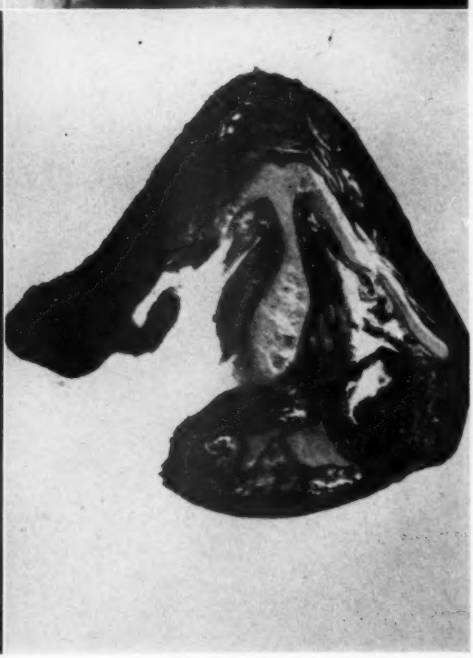

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Stupka

Etiology of Lateral Nasal Clefts

PLATE 156

FIG. 5. Enlarged photograph of the eighth serial section through the lateral nasal cleft. The upwardly turned club-shaped mass and the tissue formation above it are the remains of amniotic bands.

FIG. 6. The 27th serial section shows the marked curvature of the lateral nasal cartilage on the malformed side, beyond the region of the tissue defect.

FIG. 7. In the 49th serial section approximate bilateral symmetry has been achieved, and all structures proper to this plane show normal development.

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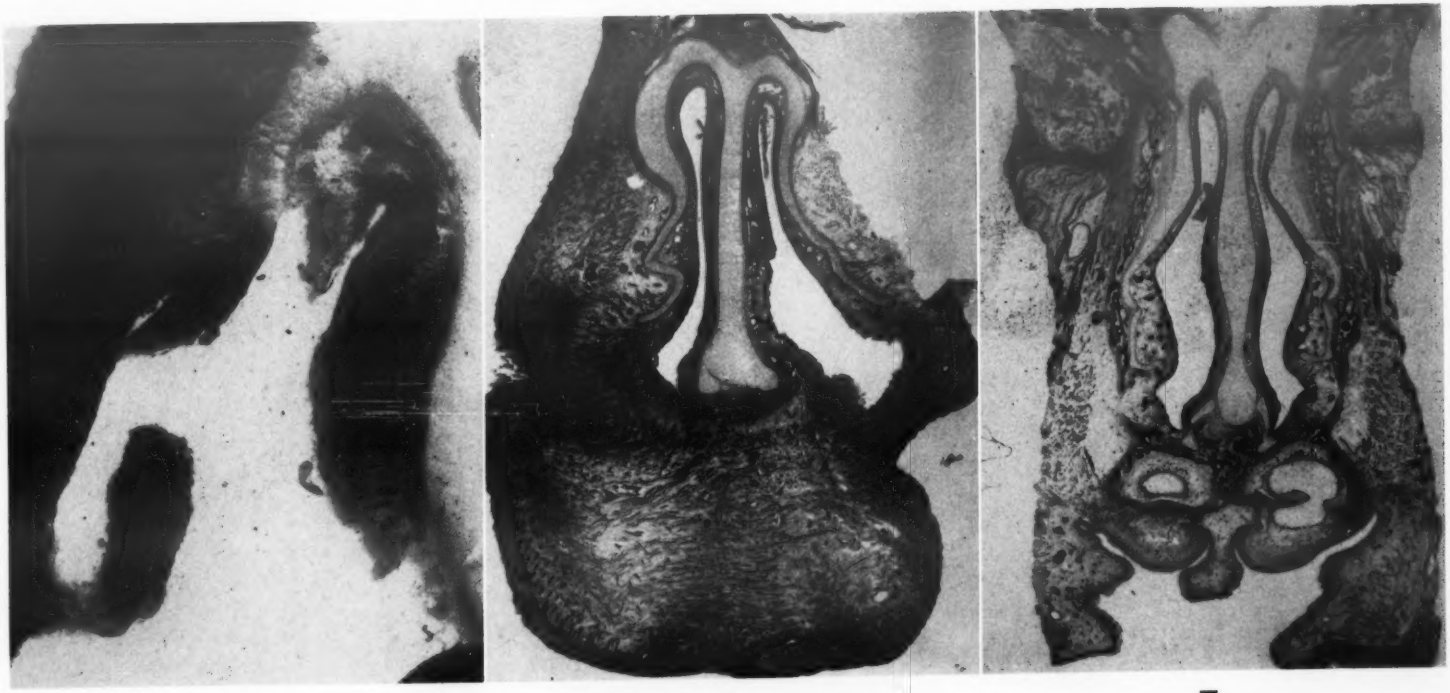

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Etiology of Lateral Nasal Clefts

OBSERVATIONS ON THE REDUCTION OF TRIPHENYL TETRAZOLIUM CHLORIDE BY NORMAL AND MALIGNANT HUMAN TISSUE *

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The reduction of tetrazolium salts to their colored formazans by various normal and neoplastic tissues has attracted the attention of investigators since the studies of Straus, Cheronis, and Straus.[1] They reported a "differential reduction of the tetrazolium by carcinomatous tissue as compared to the rate exhibited by the surrounding tissues." Similar observations were made by Antopol, Glaubach, and Goldman[2] who reported that the immersion of tissue slices in a solution of neotetrazolium yielded intense staining in liver and kidney parenchyma, epithelial tissue, and muscle, but poor staining in fibrous tissue. It was their impression that malignant tumors (no mention of type) stained more intensely than normal parenchymal tissue. The reduction of the tetrazolium salt by tissue slices appears to reflect the reductase or dehydrogenase activity of tissues and has been used to study such enzyme activity by Kun and Abood[3] and Black and Kleiner.[4] However, the exact substrate and components of the reaction have not been defined as yet, although there is some evidence that when succinate functions as a substrate there is an increased reduction of the tetrazolium salts.

Quantitative studies by Seligman, Gofstein, and Rutenburg[5] and Black and Kleiner[4] on the differential reduction of triphenyl tetrazolium chloride by various tissues of tumor-bearing animals have been reported. Using a 1-hour incubation period, Black and Kleiner found that the reduction of the dye by tissue slices of CFW mice with spontaneous mammary tumors occurred in the following order: kidney, liver, small intestine, diaphragm, tumor, spleen. While these findings indicate that many normal tissues may possess greater dehydrogenase activity than some tumors, it does not negate the possibilities that the dehydrogenase activity of tumors may exceed that of stromal tissues or that species variations may exist. This study was undertaken to note the differences between the ability of diverse human tissues, healthy and diseased, to reduce 2,3,5-triphenyl tetrazolium chloride as evidenced by the appearance of the red formazan in localized areas. It was hoped that the definition of the dehydrogenase activity of various tissue components might provide the basis for the clinical usage of tetrazolium as an aid in tumor diagnosis.

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Material and Methods

The technic employed in this study followed, with slight modifications, that of Straus, Cheronis, and Straus.[1] Free-hand slices of tissues 2 to 4 mm. thick were placed in test tubes containing 10 cc. of 1 per cent 2,3,5-triphenyl tetrazolium chloride solution buffered to pH 7.2, and were incubated in this solution at 37° C. for 30 minutes. They were then fixed in 20 per cent formalin which did not alter the color of the formazan but did destroy the color of the blood, thus removing that source of error. Variations in the color of the different organ slices and their component parts were then evaluated.

Tissue specimens were obtained from both surgical and autopsy material and it was found that with this technic no significant differences in color production occurred in tissue slices from organs removed several hours after death as compared to fresh operating-room material. The variations in color were distinct and could be defined quite as well as red or colorless. Cases with minimal coloration were included in the latter group.

Results

Marked differences in the degree of reduction of the tetrazolium were found among the different organs, and within any organ type further differences were observed among the tissue components of the organ. However, no individual variations were seen grossly between different specimens of the same tissue and organ type. Thus, a deep red color was observed in the ducts of all the breast cases tested, while the fibrous and fatty tissue failed to show appreciable color in any case.

Cytologic observations of scrapings from normal and malignant tissues indicated that the formazan was localized exclusively in the cytoplasm while the nuclei remained colorless. In some cases, the formazan appeared as minute granules while in others there was a suggestion of diffuse staining throughout the cytoplasm without any formazan crystal formation. In some cells, there appeared to be an accumulation of the dye in lipid vacuoles, which is not unexpected in view of the solubility of formazan in lipid solvents. These findings confirm the observations of Antopol *et al.*[2] As yet, no significant difference has been observed in the cytologic manifestations of the dehydrogenase activity between normal and malignant cells although this line of study cannot be considered as closed.

The organs and tissues studied may be divided into two groups on the basis of their dehydrogenase activity: those with a high degree of activity as evidenced by the production of the red formazan, and those with a lower or minimal dehydrogenase activity manifested by an ab-

sence or minimal amount of color produced. Minimal coloration was found in the following locations in various normal and diseased tissues: the submucosa, muscularis, and serosa of the gastro-intestinal and urinary tracts, fibrous and fatty tissue of the breast, all layers of the aorta, bone and cartilage, renal medulla, normal lymph node, lung, spleen, and myometrium. Minimal reaction was observed also in chronic inflammatory masses, in lymph nodes of Hodgkin's disease and chronic lymphatic leukemia, and in leiomyomatous tissue.

A deep red color was produced in the following locations in various normal organs: the mucosa of the gastro-intestinal and urinary tracts; uterine endometrium, hepatic parenchyma, renal cortex, ducts and adenomatous areas of the breast and prostate, and the parenchyma of the testis. The following malignant tumors also reduced the tetrazolium solution markedly: carcinoma of the urinary bladder, breast, esophagus, stomach, and skin, as well as acute myeloblastic leukemia, reticulum cell sarcoma, and multiple myeloma. The high reductase activity was observed both in areas of primary cancer and in the metastatic foci in lymph nodes.

It is thus evident that most of the epithelial structures tested had a high degree of dehydrogenase activity. When malignant tumors arose from such tissues, this activity was maintained in the malignant growths, such as carcinoma of the breast, stomach, and rectum, and such activity persisted in both the primary invasive process and the metastatic lesions. It is interesting to note, also, that active reduction of the tetrazolium occurred in lymph nodes from a case of acute myeloblastic leukemia and reticulum cell sarcoma, while the normal nodes and those from cases of Hodgkin's disease and chronic lymphatic leukemia failed to reduce the dye appreciably. These findings paralleled those of Roskelley, Mayer, Horwitt, and Salter [6] who found that in regard to the stimulation of respiration by succinate, lymph nodes from Hodgkin's disease behaved like normal tissue while those from lymphosarcoma behaved like malignant tissue and gave minimal succinate response.

It should be pointed out that the production of the red color in a tissue with a high degree of reductase activity usually was evident after 10 to 11 minutes of incubation. It has been our experience that the reaction may be accelerated by exposure of the tissue slice in tetrazolium to bright sunlight or to the most intense light of the Leitz microscope lamp for 3 minutes. The use of this procedure will result in the development of color in about 5 minutes, thus comparing favorably with the time required for the routine frozen section examination of tissues. The failure of inflammatory lesions of the breast to reduce the

tetrazolium appreciably in contrast to the distinct staining of diffuse invading carcinoma has proved an aid in diagnosis in those lesions difficult to evaluate by the frozen section technic.

The marked difference in the appearance of sections of breast tissue from normal breast, cystic disease of the breast, and carcinoma deserves comment. In normal breast sections, the fibrous tissue stroma appeared as a white background throughout which were scattered punctate areas of red, indicating the location of the ducts. With the advent of cystic or adenomatous changes, these punctate areas might be more numerous or larger but in no case did they lose the distinct separation from the unstained stroma and fat. In the cases of mammary carcinoma studied, the distinct architectural integrity was lost and in its place was found solid red staining, the outlines and intensity of which reflected the invasion of the stroma and replacement of parenchyma by the malignant cells. In lesions having an intense desmoplastic reaction, the color might be less intense than in growth of the medullary type but, in both, the loss of architectural definition and diffuse staining throughout the stroma was ample evidence of the nature of the process and in excellent agreement with the microscopic appearance upon histologic examination.

This alteration in the normal architectural patterns of the tissue components of an organ in the presence of cancer was evident also in other areas than the breast, *i.e.*, skin, stomach, rectum, esophagus. In all cases studied, the invading lesion was distinctly delineated by the diffuse red color produced by the tumor cells in areas that should normally have minimal color after incubation with the tetrazolium solution. Such characteristic alterations have been noted even in cases of tumors no larger than 3 mm. in diameter. Despite the distinct pattern produced in tissues upon the advent of cancer, it was not our impression that the malignant tissue reduced the dye more readily than the tissue of origin under the conditions used. In general, the reverse would appear to be more usual.

The color reaction of lymph nodes which had been invaded by carcinoma was in distinct contrast to that of normal nodes. The dehydrogenase activity of the latter was not great as evidenced by minimal red color produced with the technic employed. When carcinomatous metastases occurred the neoplastic tissue was distinctly defined by the localization of the formazan, and complete replacement of the nodal parenchyma by neoplastic tissue was associated with a diffuse red color throughout. When the metastatic invasion was confined to a focal area,

this was indicated by the red staining neoplastic tissue in contrast to the surrounding unstained or pink normal nodal parenchyma.

Discussion

The data presented are of interest from two points of view: (1) The use of the tetrazolium dyes appears to constitute another tool for studying vital metabolic processes in a variety of cells and tissues both normal and malignant, and (2) the color formazan produced by the carcinomatous tissue provided an excellent topographic demonstration of invasion into the underlying non-staining stroma. The latter aspect alone has proved helpful as an aid to the frozen section method of tissue examination when a rapid decision is needed as to the malignancy or benignancy of a growth. In addition, it provides excellent visualization of metastatic spread to lymph nodes, particularly where such nodes are only partially involved. Further, the minimal reaction in normal nodes and in nodes of Hodgkin's disease and chronic leukemia contrasted with the red color of acute myeloblastic leukemia and reticulum cell sarcoma may provide an interesting point of departure for further study of these conditions. While this observation appears to be in accord with the findings of Greenstein [7] that enzyme activities which are low in normal tissues tend to rise on the advent of malignant transformation, further investigation will be needed to clarify this possibility.

Summary

Tissue slices from a variety of human organs and tissues were incubated in a solution of 2,3,5-triphenyl tetrazolium chloride and the relative amounts and localization of the red formazan were noted. With this technic it was found that:

1. Triphenyl tetrazolium chloride is actively reduced by most epithelial elements of most tissues and to a much less degree by normal lymphoid or fibrous tissue or by inflammatory tissue.

2. Malignant tumors arising from epithelial structures tend to retain the high dehydrogenase activity of the parent tissue.

3. The high degree of contrast between the colored malignant tissue and the unstained stroma or lymph node provides an excellent topographic demonstration of tumor spread.

4. The ability to accelerate the reaction by light makes the procedure practical as an aid for the frozen section technic in determining the presence of a malignant neoplasm.

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STRUCTURE AND IODINE CONTENT OF THYROTOXIC GOITERS IN ICELAND *

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No other organ is so variable in respect to size and histologic structure, within the limits of what is regarded as normal, as is the thyroid gland. No wonder that the pathologic changes superimposed on this variable "normal" state may display the greatest diversity.

Although the literature contains abundant descriptions of the histologic structure of goiter, toxic and nontoxic, from various parts of the world, it was thought that some account of the material seen in Iceland would still be relevant.

In Iceland the human thyroid gland is unusually small. The average size in the adult male has been found to be about 14 gm. and in the female, 11.6 gm.[1] This is only about one-half the "textbook-size" for normal thyroid glands in non-goitrous countries. It was found also that the Icelandic thyroid gland is exceptionally rich in iodine calculated per unit weight, containing 4.01 and 3.43 mg. of iodine per 1 gm. of dry substance as averages for adult males and females, respectively. The total amount of iodine, however, was within the range of what has been found elsewhere for normal thyroid glands.[1,2] The epithelium in the normal state is flattened or at most low-cuboidal, the acini large and as a rule distended with deeply stained colloid, so that, in general, the histologic picture approaches that of a resting colloid gland in the sense of Marine.[3,4]

The statement of Marine[3,4] that hyperplastic changes do not occur in glands of normal size so long as the iodine content is above 1 per cent of the dried weight of the gland, was not found to hold true for the small thyroid glands in Iceland. In fact the relative iodine content of the adult thyroid gland was never found to be below this level and evidence of hyperplastic changes could be seen at much higher levels of iodine concentration. On the whole there appeared to be no definite level of relative iodine content which could be taken to be decisive for the presence or absence of hyperplasia. However, such a level, or rather borderline zone, could be accepted in terms of total iodine content; and some arguments have been advanced to show that in order to give general applicancy to the well known and widely accepted theory of Marine, explaining how the thyroid cell cycle is regulated by the iodine

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content, it must be modified to the effect that it is the total and not the relative iodine content which is the deciding factor.[1,2]

Briefly then, the lower the iodine concentration, the larger the gland must be to be able to store the necessary amount of iodine. As the iodine concentration, however, is dependent on the amount of iodine intake, it is the latter that ultimately decides the size of the thyroid gland. In Iceland the iodine intake of the population is undoubtedly very high because of the great consumption of fish and fish products. This leads to the unusually high concentration of iodine in the thyroid gland which again results in the smallness of that organ.

As is to be expected from the foregoing, endemic goiter does not occur in Iceland nor is it ever known to have occurred, and sporadic cases of simple goiter are rare. Hyperthyroidism, including exophthalmic goiter, however, appears to be relatively frequent. People are rather thyroid conscious and only a slight enlargement is required to cause complaints of local pressure symptoms or of unsightliness. Of the non-toxic goiters thus presented for surgical treatment, the great majority have proved to consist of circumscribed solitary nodules.

Material

The present material, most of which dates from the years 1944-1947, consists of unselected cases of surgically removed goiters. In most cases the whole part removed was first sent to the Pathological Institute in Reykjavik where a small portion was excised for histologic diagnosis. One or more slices were taken for histologic examination (paraffin sections stained with hematoxylin and eosin and with van Gieson's stain); then a suitable portion, depending on the homogeneity of the goiter, was minced, dried at 50°C., and pulverized.

Iodine Estimation. Two-tenths gm. of the dried pulverized gland was combined in a nickel crucible with 10 gm. of the following mixture: potassium nitrate, 125 gm.; desiccated potassium carbonate, 230 gm.; and desiccated sodium carbonate, 177 gm. It was then covered with 5 gm. of the same mixture and ashed in an electric muffle at about 450° C. The ashing being completed, the melt was dissolved in boiling water, neutralized with sulfuric acid diluted with two parts of water, methyl orange serving as indicator, a few drops of the acid being added in excess. After the solution had been boiled gently for 10 minutes, saturated bromine water (freshly made) was added until a faint brownish color remained. Then the solution was boiled gently again to expel the excess of bromine. After cooling, a few drops of 10 per cent solution of potassium iodide were added and the liberated iodine titrated with N/100 sodium thiosulfate. (For further details see Sigurjonsson, 1938.[5])

Of the total of 70 cases collected, 50 were diffuse goiters (including 2 diffuse and nodular). The remaining 20 were nodose, *i.e.*, they consisted of one circumscribed node, except in 2 instances where there

were two, one in each lobe. All but 2 of the diffuse goiters were from patients suffering from thyrotoxicosis and they had nearly all received iodine as preoperative treatment (Table II). Of the 20 cases of nodose goiters, 5 were associated with hyperthyrosis. The size of the goiters varied from 8 to 168 gm., the majority being below 40 gm. as seen from Table I. (See also Tables II and III.)

TABLE I

Size of Goiters

Weight	Diffuse goiters		Nodose goiters
	Number with Graves-Basedow's disease	Number with hyperthyrosis	Number
Below 10 gm.		1	1
10–19 gm.	5	3	4
20–29 gm.	16		2
30–39 gm.	6		2
40–59 gm.	6		
60–79 gm.	1	1	3
80–99 gm.	2		
Above 100 gm.		2	
Total	36	7	12

Histologic Findings

Since the introduction of preoperative iodine treatment in hyperthyroidism, the classical histologic picture of the Graves-Basedow goiter (exophthalmic goiter) is rarely seen, the general change in appearance being toward that seen in an ordinary colloid goiter with or even without hyperplasia. This was clearly seen in the present study of thyrotoxic goiters. At the one extreme were cases largely of parenchymatous appearance with very little colloid and tall hyperplastic epithelium filling the acini or projecting into their lumina. At the other extreme was the appearance of a typical resting colloid gland. Between these extremes all grades of hyperplastic changes in a more or less colloid-rich gland were seen.

Apart from the cases of predominantly parenchymatous hyperplastic type, hyperplastic changes of two other types were distinguished: (1) Papillary projections, lined by high epithelium, into the lumen of the acini. These projections often showed cauliflower-like structures, as has been described frequently, especially by German writers. (2) A mere inbulging of the acinar wall at places where the epithelium was high, sometimes displaying new formation of acini at the base, in which case the picture might be that of a "Sanderson's Polsterung."

Although no presumption was made that these differences in appearance represented more than different stages of involution effected by the iodine therapy or different stages of hyperplasia in general, they were adopted as a base for preliminary grouping, keeping also in mind

that it was the latter form only (non-papillary hyperplasia) which was seen in an otherwise normal material in this environment.

Proceeding on these lines, the diffuse goiters were grouped according to the histologic picture as follows:

(1) Predominantly of parenchymatous hyperplastic structure al-

TABLE II

Diffuse Goiters

No.	Sex and age	Exophthalmos	Basal metabolic rate*	Preoperative iodine	Microscopic appearance	Lymphoid nodules	Iodine per gm. dry gland	Weight of goiter
							mg.	*gm.*
					Graves-Basedow's disease			
1	F 32	+	26	+	Parenchymatous hyperplasia, Basedow type, colloid sparse	+	1.153	25
2	F 52	+	86	+†	Parenchymatous hyperplasia, Basedow type, colloid sparse	+	0.888	23‡
3	F 32	+	69	+	Parenchymatous hyperplasia (Basedow type), signs of involution	+	0.734	28
4	F 23	+	45	+	Parenchymatous hyperplasia (Basedow type), signs of involution	+	1.000	45.5
5	F 47	+	60	+	Mostly parenchymatous hyperplasia (Basedow type), signs of involution	+	0.903	50
6	F 51	+	28	+	Mostly parenchymatous hyperplasia (Basedow type), signs of involution	+	1.174	16‡
7	F 44	+	32	+	Papillary hyperplasia, moderate colloid storage	+	1.260	90
8	F 24	+	46	+†	Papillary hyperplasia, moderate colloid storage	−	1.627	27.5
9	M 24	?	62	+†	Papillary hyperplasia, varying amounts of colloid	+	0.601	84
10	F 31	+	32	+	Papillary hyperplasia and colloid hyperplasia	−	1.883	21
11	F 29	+	39	+	Papillary hyperplasia, parenchymatous and colloid-rich areas	+	1.584	25
12	F 31	+	87	+	Papillary hyperplasia and colloid hyperplasia	−	1.734	39
13	M 40	+	86	+	Papillary hyperplasia, moderate colloid storage	+	2.548	44.5
14	F 36	−	66	+	Papillary hyperplasia, moderate colloid storage	+	4.040	24
15	F 45	−	42	+	Patchy papillary hyperplasia and colloid-rich areas	+	2.327	52‡
16	F 41	+	63	+	Patchy papillary hyperplasia and colloid-rich areas	+	1.197	27
17	F 33	+	67	+	Patchy papillary hyperplasia and colloid-rich areas	−	3.426	28
18	F 41	?	110	+	Papillary hyperplasia in colloid gland	+	3.556	30‡
19	F 29	+	46	+†	Papillary hyperplasia in colloid gland	+	2.251	23‡
20	M 30	+	47	+	Patchy papillary hyperplasia in colloid gland	−	2.517	26.5
21	F 36	+	51	+	Patchy papillary hyperplasia in colloid gland	+	2.679	27
22	F 22	+	50	+	Colloid hyperplasia, some papillary projections	−	1.320	23
23	F 25	+	48	+	Colloid hyperplasia, some papillary projections	+	2.665	30
24	F 36	+	59	+	Colloid hyperplasia, a few papillary projections	+	2.866	67
25	F 28	+	34	+	Colloid hyperplasia, a few papillary projections	+	2.163	25
26	F 21	+	+	+	Normal colloid hyperplasia, a few papillary projections	+	1.328	
27	F 26	+	42	+	Colloid hyperplasia, papillary projections	−	2.327	19
28	M 38	+	49	+	Patchy hyperplasia and papillae in colloid-rich gland	+	4.089	57
29	F 51	+	45	+	Hyperplasia in colloid-rich gland	+	1.675	
30	F 48	−	40	+	Colloid hyperplasia	+	3.013	21
31	F 30	−	56	+†	Colloid gland with slight hyperplasia	+	2.887	42
32	F 33	−	45	+	Colloid gland with slight hyperplasia	−	2.317	17
33	F 36	+	75	+	Colloid gland with slight hyperplasia	+	2.016	13.5
34	M 58	+	70	+	Colloid gland with slight hyperplasia	+	5.626	37‡
35	F 24	−	71	+	Colloid gland (without hyperplasia)	−	3.177	15
36	F 31	+	42	+	Colloid gland (without hyperplasia)	−	4.198	27
37	F 25	+	45	+	Micro-acini, sparse colloid, active epithelium, hyperplasia	+	0.465	30
38	F 22	+	54	+	Active epithelium, hyperplasia moderately colloid-rich	+	0.939	34.5
					Hyperthyrosis			
39	F 27	−	34	+	Normal colloid hyperplasia	−	2.807	8
40	F 30	−	?	+	Normal colloid hyperplasia	+	1.481	−
41	F 19	−	21	+	Colloid hyperplasia	−	1.829	10‡
42	F 37	−	29	+	Normal colloid gland	−	1.735	
43	F 31	−	46	+	Colloid gland	−	2.988	13
44	M 28	+	+	−	Colloid gland	−	4.061	
45	F 61	−	38	+	Micro-acini, epithelium cuboidal	−	0.665	74
46	F 54	−	62	+	Micro- and macro-acini, active epithelium	+	0.534	168
47	F 38	+	?	?	Nodular, colloid-rich, micro-cysts, slight hyperplasia	−	2.030	15
48	F 41	−	30	?	Nodular, colloid-rich, micro-cysts	−	0.652	103
					Euthyrosis			
49	F 38	−	−20	−	Colloid gland, active	−	0.317	50
50	F 39	−	?	−	Active gland	−	0.435	

* Highest value recorded before operation (percentage above normal).

† Methylthiouracil also administered before operation.

‡ Weight after fixation in formol and after removal of one block for histologic diagnosis.

though some colloid containing acini may be seen at places. Epithelium hyperplastic, sometimes showing plications.

(2) Widespread papillary hyperplasia in a fairly colloid-rich gland.

(3) Colloid-rich glands with hyperplasia mainly of the non-papillary type, but also showing traces of papillary projections.

TABLE III

Solitary Nodules

No.	Sex and age	Exophthalmos	Basal metabolic rate*	Preoperative iodine	Microscopic appearance	Lymphoid nodules	Iodine per gm. dry gland	Weight of goiter
							mg.	*gm.*
					Hyperthyrosis			
1	F 33	–	38	–	Parenchymatous (fetal adenoma)	–	0.220	
2	F 41	–	36	–	Parenchymatous (fetal adenoma)	–	0.106	
3	F 50	–	25	+	Parenchymatous and micro-acinar, hemorrhages	–	0.097	73†
4	F 49	–	49	+	Colloid nodule, active epithelium, hemorrhages	+	1.620	26†
5	F 60		22	+	Colloid nodule, micro-cysts, proliferation, hemorrhages	–	1.861	14.5†
					Euthyrosis			
6	F 38	–		–	Parenchymatous (fetal adenoma)	–	0.021	10
7	F 28	–	8	–	Parenchymatous (fetal adenoma)	–	0.212	
8	F 60	–		–	Parenchymatous and micro-acinar (fetal adenoma)	–	0.602	
9	F 53	–			Parenchymatous and micro-acinar (fetal adenoma)	–	0.467	10
10	F 43	–	−12	–	Parenchymatous and one hemorrhagic cyst	–	0.106	30
11	F 46	–	19	–	Normal colloid structure	–	1.587	36
12	F 34	–		–	Micro-cystic colloid nodule, degeneration and hemorrhages	–	0.384	16†
13	F 29	–		–	Colloid nodule, degenerative changes	–	0.032	
14	F ?	–		+	Colloid nodule, micro-acini, hyaline degeneration	–	0.703	63†
15	F 42	?	11	?	Colloid nodule, micro-acinar and cystic, hemorrhages	–	0.063	63
16	F 44	–		?	Cysts (two)	–		23.5†
17	F 54	–	5	–	Cyst	–		
18	M 43	–			Cyst			
19	M 39	–		–	Papilloma (two nodules)	–	0.045	
20	F 46	–		–	Adeno-papilloma	–	0.825	5†

* Highest value recorded before operation (percentage above normal).

† Weight after fixation in formol and after removal of one block for histologic diagnosis.

(4) Colloid glands showing a slight degree of non-papillary hyperplasia.

(5) Colloid glands in a resting state.

These five groups might be taken as representing different stages in the same cycle of changes.

(6) Active microfollicular glands, *i.e.*, with small, rounded acini containing some colloid, the epithelium being high-cuboidal or higher (active epithelium), sometimes showing inbulging at places but no papillary projections.

(7) Diffuse and nodular goiter. The enlargement was macroscopically diffuse, but the histologic structure was nodular. These glands were on the whole colloid-rich and the nodes, which were not sharply demarcated, consisted of groups of enlarged acini distended with colloid or colloid cysts. Degenerative changes were seen in some of the nodules.

The nodose goiters were grouped as follows:

(1) Parenchymatous nodules of the "fetal adenoma" type, often showing at places small colloid-containing acini.

(2) Colloid nodules of irregular structure, often showing cystic acini, degenerative changes, and extensive hemorrhages.

(3) Solitary cysts with semifluid content. In these cases, since the cysts had been opened and the content lost, iodine estimation could not be carried out.

(4) Papilloma or adenopapilloma.

Clinical History

According to the clinical manifestations as revealed by the case histories, the following three groups were distinguished: Graves-Basedow's disease, simple hyperthyrosis, and nontoxic goiters (euthyrosis). This grouping, which was performed independently of the histologic examination, was in most instances easy enough, but there were a few cases in which opinion might have differed as between Basedow's disease or hyperthyrosis, or again as between mild hyperthyrosis or non-toxic goiter.

The term hyperthyrosis as used here comprises all cases of thyrotoxicosis which do not conform wholly to the clinical syndrome of Graves-Basedow's disease, but resemble more closely the condition resulting from excessive dosage of thyroxin. Accordingly, most cases of the so-called toxic adenomas fall within this group. Among others, Josselin de Jong,[6] who differentiated in the same way between Morbus Graves-Basedow and hyperthyrosis, was of the opinion that there is a fundamental difference between these two clinical forms of thyrotoxicosis. This has been questioned, however, as the difference seems not to be such that it could not be explained by varying intensity of toxicosis and a different constitutional type of the individual. Also there is no single criterion which can be absolutely relied upon for the differentiation. Exophthalmos, for instance, although characteristic for Graves-Basedow, may be absent, at least at some stages of the disease (in my series it was found absent in 6 cases, or in about 16 per cent); on the other hand, it may occur in simple hyperthyrosis, although rarely. Nor do the histologic findings—characteristic as they are in many cases of Graves-Basedow's disease—allow a clear separation of the two clinical forms. It has been pointed out that the age distribution, especially of the so-called toxic adenomas, is different from that of Graves-Basedow's disease which occurs at an earlier age. But it must be remembered that the incidence of nodose goiters in general increases with age. In the Icelandic material there was no difference in this respect between the groups of Graves-Basedow's disease and of hyperthyrosis associated with diffuse enlargement. In the former group of 38 cases, 34 per cent were below 30 years and 71 per cent below 40 years of age; in the latter

group of only 10 cases the corresponding figures were 30 and 70 per cent. However, the cases are too few to impart much significance to this comparison.

In Tables II and III are shown the results of the histologic examination and iodine determination in each of the individual cases. From these tables, Table IV is compiled showing the twofold grouping, *i.e.*, according to structure and clinical aspect, as well as the average and range of iodine content for each group.

Discussion

As is seen from Table IV, 38 of the 50 diffuse goiters were classified as Basedow's disease and 10 as simple hyperthyrosis. Comparing the histologic findings, it is seen further that in the majority of the Basedow cases hyperplasia of the papillary type or diffuse parenchymatous hyperplasia has been found (groups 1 to 3, Table IV), but in none of the other cases. The remaining Basedow cases do not differ histologically from the cases of simple hyperthyrosis or euthyrosis. From this it

Table IV

Combined Clinical, Morphologic, and Chemical Grouping

Histologic character	Graves-Basedow's disease				Hyperthyrosis				Euthyrosis			
	Iodine, mg./gm. dry weight				Iodine, mg./gm. dry weight				Iodine, mg./gm. dry weight			
	Number	Min.	Max.	Average	Number	Min.	Max.	Average	Number	Min.	Max.	Average
Diffuse goiters:												
Predominantly parenchymatous hyperplasia	6	0.73	1.17	0.97								
Papillary hyperplasia, varying amounts of colloid	15	0.60	4.04	2.22								
Hyperplasia, some papillary projections, fairly colloid-rich	7	1.32	4.09	2.40	3	1.48	2.81	2.04	2	0.32	0.44	0.38
Slight hyperplasia, colloid-rich	6	1.68	5.63	2.93	3	1.74	4.06	2.93				
Colloid gland (resting)	2	3.18	4.20	3.69	2	0.53	0.67	0.60				
Small acini, active epithelium, sparse colloid	2	0.47	0.94	0.71	2	0.65	2.03	1.34				
Diffuse and nodular goiter									2			
Total	38				10				2			
Solitary nodes:												
Parenchymatous (fetal adenoma)					3	0.11	0.70	0.34	5	0.02	0.60	0.28
Colloid nodes (often with hemorrhages and degeneration)					2	1.62	1.86	1.74	5	0.03	1.59	0.55
Cysts									3			
Papilloma and adenopapilloma									2	0.05	0.83	0.44
Total (nodes)					5				15			

appears that here the papillary form of hyperplasia can be regarded as fairly characteristic for exophthalmic goiter, but when absent in an iodine-treated gland, no inference can be drawn from the histologic picture as to the clinical function in a single case, although the presence of lymphocytic follicles speaks for hyperfunction. In the Graves-Basedow goiters, lymphoid nodules were found in 74 per cent of the cases but in only 2 of the 10 cases of simple hyperthyrosis and in none of the atoxic goiters. Five of the 38 Graves-Basedow patients and one of the patients with simple hyperthyrosis were males.

In regard to the diffusely enlarged glands, there is apparently some correlation between the amount of colloid, as judged from the histologic examination, and the iodine content. The first group in Table IV, those with parenchymatous hyperplasia, showed only a slight amount of iodine (average, 0.97 mg./gm. dry weight) although considerably more than was commonly found before the introduction of preoperative iodine treatment.[7,8] In the cases in two succeeding stages of involution, in which colloid is becoming fairly abundant, the iodine content had risen considerably, averaging 2.22 and 2.40 mg., respectively. These two groups did not differ markedly in respect to colloid content, the difference in microscopic appearance lying mainly in the extent of hyperplastic changes. With increasing storage of colloid and diminishing signs of hyperplasia, there was a further rise in the iodine content which reached the highest value in resting colloid glands. In spite of the iodine medication, however, the iodine content of these glands did not exceed that seen in normal glands in this locality. It is noteworthy that the two non-toxic diffuse goiters, in spite of being rich in colloid and showing only slight hyperplasia, were markedly iodine poor, but to these patients no iodine had been administered before operation. Only 5 of the patients had been given methylthiouracil before operation but all had received iodine as well, and this is probably the reason why the effect of thiouracil is not reflected in the histologic appearance.

Solitary Nodules

Of the 20 cases with solitary nodules, varying in size from about 10 to 70 gm., 2 were definite tumors (papillomas) and 3 cysts. The remaining 15 were classed as parenchymatous or colloid nodules. Neither histologic examination nor iodine estimation revealed any difference between the toxic nodules (which were 5) and the non-toxic ones.

The parenchymatous nodules were of the character of the so-called fetal adenomas of Wölfler,[9] showing solid epithelial cell groups or small

acini without colloid, although at some places a more distinct alveolar structure with small rounded, colloid-containing acini might be seen. From the histologic picture it seems reasonable to assume that such a nodule has originated from a "fetal adenoma" which, instead of remaining quiescent as the small whitish nodules frequently seen in otherwise normal glands, has in some way acquired an autonomous growth and established itself as a true tumor.

The structure of the colloid nodules was more variable. In general they appeared as a colloid gland with or without hyperplasia, but the acini were irregular in size and shape and often enlarged so as to appear cystic. Degenerative changes and hemorrhages were frequent. In cases in which degenerative changes or hemorrhages were extensive, the iodine content was very low; otherwise it was higher than in the parenchymatous nodules, although much lower than in normal glands. It was not evident that iodine administered before operation had increased the iodine storage (Table II).

The terminology relating to toxic goiters is rather confused, and distinction is not always made between clinical and morphologic terms. From the clinical point of view one can make a distinction between genuine Graves-Basedow's disease and simple hyperthyrosis, and one can also, based on morphologic criteria, distinguish diffuse and nodular goiters, but these two modes of grouping do not cover each other.

The separation into Graves-Basedow's disease and toxic adenoma cannot be used as a binary classification of toxic goiters as it comprises neither diffuse goiters with simple hyperthyrosis nor nodose goiters showing the syndrome of Graves-Basedow. These two forms may be relatively infrequent in some regions but in the large material described by Josselin de Jong,[6] for instance, they were by no means rare, and in the material presented here simple hyperthyrosis was more often associated with diffuse than with nodose goiter. The differentiation of primary and secondary toxicosis is also of limited applicability, at least as primary classification. There is no doubt that typical Basedow changes can be superimposed on goitrous enlarged glands; on the other hand there is not always evidence of non-toxic goiter preceding symptoms of simple hyperthyrosis. In 2 or 3 of the 38 cases presented here as Graves-Basedow's disease there was an enlargement of the gland noted by the patients before the onset of symptoms, and of the 10 patients with hyperthyrosis associated with diffuse enlargement 7 stated that noticeable enlargement and the clinical symptoms had occurred almost simultaneously.

It is highly desirable that a more uniform terminology be adopted and there seems to be some practical advantage in classifying primarily according to the clinical picture, as has been done here, and then, secondarily, according to the morphologic findings.

Summary

Diffuse goitrous enlargement of the thyroid gland is rarely seen in Iceland except when accompanied by thyrotoxicosis. Thus, of 50 diffuse goiters examined, 38 (76 per cent) were associated with Graves-Basedow's disease and 10 (20 per cent) with simple hyperthyrosis, while only 2 (4 per cent) were considered to be without definite symptoms of thyrotoxicosis.

On microscopic examination, 28 of the 38 cases of Graves-Basedow's disease showed characteristic epithelial changes but the remaining 10 could not be differentiated from the cases of hyperthyrosis or even euthyrosis. In 5 cases of thyrotoxicosis there was no evidence of epithelial hyperplasia. Lymphoid nodules were seen in 74 per cent of the Graves-Basedow and in 20 per cent of the hyperthyrotic glands.

Of 20 nodular goiters—the enlargement being caused by one or at most two circumscribed masses—only 5 were associated with some toxicosis. Histologically, 2 of the nodules were papillomas, 3 cysts, 8 parenchymatous (fetal) adenomas, and 7 colloid nodules with degenerative changes and hemorrhages. Those associated with toxicosis (3 parenchymatous and 2 colloid nodules) could not, from the histologic picture, be differentiated from the non-toxic nodules of the same groups.

The relative iodine content of the diffuse goiters corresponded roughly to the richness in colloid. In only a few cases did it reach or surpass the normal average in spite of preoperative iodine treatment. On the whole, the nodules were iodine poor.

It is pointed out, as has been shown elsewhere, that the thyroid cell cycle is not regulated so much by the relative iodine content (as maintained by Marine[3,4]) as by the total iodine amount.

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* Abstract of paper presented at the meeting of The American Association of Pathologists and Bacteriologists held at Madison, April 13, 14, and 15, 1950.

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